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1.	Your reference	SCB/NLW/60515/000	24SEP02 E750429-1 D02882 P01/7700 0.00-0222077.0
2.	Patent application number *(The Patent Office will fill in this part)*	0222077.0	23 SEP 2002
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	**GW PHARMA LIMITED** **PORTON DOWN SCIENCE PARK** **SALISBURY** **WILTSHIRE** **SP4 0JQ**	
	Patents ADP number *(if you know it)*		
	If the applicant is a corporate body, give the country/state of its incorporation	**UNITED KINGDOM**	8316945001
4.	Title of the invention	**METHODS OF PREPARING CANNABINOIDS FROM PLANT MATERIAL**	
5.	Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* Patents ADP number *(if you know it)*	**BOULT WADE TENNANT** **VERULAM GARDENS** **70 GRAY'S INN ROAD** **LONDON WC1X 8BT** **42001**	

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number (if you know it)	Date of filing *(day/month/year)*

7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing *(day / month / year)*

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	**YES**

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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(Please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature: Boult Wade Tennant

Date: 23 September 2002

12. Name and daytime telephone number of person to contact in the United Kingdom: **Nina L White 020 7430 7500**

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Methods of preparing cannabinoids from plant material

Field of the invention

The invention relates to methods of preparing cannabinoids in substantially pure form starting from plant material.

Background to the invention

Cannabis has been used medicinally for many years, and in Victorian times was a widely used component of prescription medicines. It was used as a hypnotic sedative for the treatment of "hysteria, delirium, epilepsy, nervous insomnia, migraine, pain and dysmenorrhoea". Historically, cannabis was regarded by many physicians as unique; having the ability to counteract pain resistant to opioid analgesics, in conditions such as spinal cord injury, and other forms of neuropathic pain including pain and spasm in multiple sclerosis.

The use of cannabis continued until the middle of the twentieth century, when the recreational use of cannabis prompted legislation which resulted in the prohibition of its use. The utility of cannabis as a prescription medicine is now being re-evaluated. The discovery of specific cannabinoid receptors and new methods of administration have made it possible to extend the use of cannabis-based medicines to historic and novel indications.

The principle cannabinoid components present in herbal cannabis are the cannabinoid acids Δ^9 tetrahydrocannabinolic acid (Δ^9 THCA) and cannabidiolic acid (CBDA), with small amounts of the corresponding neutral cannabinoids, respectively Δ^9 tetrahydrocannabinol (Δ^9 THC) and cannabidiol (CBD). Cannabidiol was formerly regarded as an inactive

constituent, however there is emerging evidence that it has pharmacological activity, which is different from that of Δ^9 THC in several respects.

In addition to these major cannabinoids, herbal cannabis may contain lower levels of other minor cannabinoids. These may be intermediates in the biosynthesis of the major cannabinoids and hence exist at only low levels in the plant as they are constantly undergoing further biotransformation once they are formed. An example of such a cannabinoid is cannabigerol (CBG). Other minor cannabinoids may represent the end point of an alternative biosynthetic pathway to that leading to the formation of the major cannabinoids Δ^9 THC and CBD. These cannabinoids are frequently relatively more abundant in the plant, an example being cannabichromene (CBC).

A special example of a minor cannabinoid that is the end point of a biosynthetic pathway is Δ^9 Tetrahydrocannabivarin (Δ^9 THCV). This compound is closely related to Δ^9 THC, with the only difference in structure being the presence of a propyl (C_3H_7) side chain rather than a pentyl (C_5H_{11}) side chain on the aromatic ring. This compound usually accompanies Δ^9 THC in at a level of 1-2% of THC present. However in certain selectively bred varieties of cannabis Δ^9 THCV can account for greater than 70% of total cannabinoids, with Δ^9 THC being reduced to the level of a minor constituent.

Purified forms of certain of the cannabinoids present in herbal cannabis are useful as active pharmaceutical agents. For example, Δ^9 THC (also known as dronabinol) has been approved by the Food and Drug Administration (FDA) for the control of nausea and vomiting associated with chemotherapy, and also

shows potential pharmacological activity in the treatment of glaucoma, migraine headaches, anxiety, and as an analgesic. Cannabidiol, formerly regarded as an inactive constituent of cannabis, has, as aforesaid, itself shown promising pharmacological activity.

In the case of the minor cannabinoids, the difficulties in isolating the minor cannabinoids in a pure state and the absence of commercially available standards have restricted the investigation of the pharmacology of these compounds and their true therapeutic potential is unknown. Consequently it is of great interest to isolate sufficiently pure samples of these cannabinoids in the quantities required to permit pharmacological studies to be performed.

Purified forms of the cannabinoids and cannabinoid acids are also potentially useful as analytical standards, particularly in the characterisation of cannabis-derived medicines based on botanical drug substances prepared from herbal cannabis.

Thus, there remains a need for purified forms of all of the cannabinoid acids and cannabinoids present in cannabis herb, including the major cannabinoids Δ^9 THC and CBD and the minor cannabinoids.

Synthetic forms of certain of the cannabinoids, particularly Δ^9 THC, CBD and CBN, are commercially available. However, synthetic cannabinoids are extremely expensive. Attention has therefore focussed on the purification of cannabinoids from plant material.

US-A-6,365,416 describes a method of preparing Δ^9

THC from plant material which involves extracting the plant material with a non-polar organic solvent, optionally subjecting the extract to a column chromatography step to produce a residue eluate, subjecting the extract or the residue eluate to a low pressure flash distillation to produce a distillate, optionally subjecting the distillate to a second flash distillation step, and subjecting the distillate to column chromotography, normal HPLC or reverse-phase HPLC. The process provides a product containing Δ^9 THC in an amount greater than 90% by weight.

There remains a need for alternative purification processes which may be used to prepare purified forms of all cannabinoid and cannabinoid acid constituents of cannabis herb, including the cannabinoid acids Δ^9 THCA and CBDA, the corresponding free cannabinoids Δ^9 THC and CBD, and the minor cannabinoids. The present invention relates to such a purification process based on a simple combination of solvent extraction, chromatography and re-crystallisation steps. The process is simple, efficient and economic, and is capable of producing cannabinoids of high purity, whilst avoiding the need for preparatory HPLC.

Summary of the invention

In a first aspect the invention provides a method of obtaining a substantially pure cannabinoid or cannabinoid acid or a product enriched in a given cannabinoid or cannabinoid acid comprising:

i) obtaining an extract containing a cannabinoid or cannabinoid acid from a plant material;

ii) subjecting the extract of step (i) to a chromatographic step to produce a partially purified extract;

iii) dissolving the partially purified extract in a first solvent, removing any insoluble material

therefrom, and removing the solvent; and
iv) dissolving the product obtained in step iii) in a second solvent, removing any insoluble material therefrom, and removing the solvent to obtain the substantially pure cannabinoid or cannabinoid acid or the product enriched in a given cannabinoid or cannabinoid acid.

Description of the invention

The invention relates to a purification process for preparing substantially pure cannabinoid or cannabinoid acid or a product enriched in a given cannabinoid or cannabinoid acid from plant material.

A "substantially pure" preparation of cannabinoid or cannabinoid acid is defined as a preparation having a chromatographic purity (of the desired cannabinoid or cannabinoid acid) of greater than 95%, more preferably greater than 96%, more preferably greater than 97%, more preferably greater than 98%, more preferably greater than 99% and most preferably greater than 99.5%, as determined by area normalisation of an HPLC profile.

The term "product enriched in a given cannabinoid or cannabinoid acid" encompasses preparations having at least 80%, preferable greater than 85%, more preferably greater than 90% chromatographic purity for the desired cannabinoid or cannabinoid acid. Such a product will generally contain a greater proportion of impurities, non-target materials and other cannabinoids than a "substantially pure" preparation.

The method of the invention may be used to extract/purify cannabinoids or cannabinoid acids from any plant material known to contain such cannabinoids

or cannabinoid acids. Most typically, but not necessarily, the "plant material" will be derived from one or more cannabis plants.

The term "plant material" encompasses a plant or plant part (e.g. bark, wood, leaves, stems, roots, flowers, fruits, seeds, berries or parts thereof) as well as exudates, and includes material falling within the definition of "botanical raw material" in the Guidance for Industry Botanical Drug Products Draft Guidance, August 2000, US Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research.

The term "cannabis plant(s)" encompasses wild type *Cannabis sativa* and also variants thereof, including cannabis chemovars (varieties characterised by virtue of chemical composition) which naturally contain different amounts of the individual cannabinoids, also *Cannabis sativa* subspecies *indica* including the variants *var. indica* and *var.kafiristanica*, *Cannabis indica* and also plants which are the result of genetic crosses, self-crosses or hybrids thereof. The term "cannabis plant material" is to be interpreted accordingly as encompassing plant material derived from one or more cannabis plants. For the avoidance of doubt it is hereby stated that "cannabis plant material" includes herbal cannabis and dried cannabis biomass.

"Decarboxylated cannabis plant material" refers to cannabis plant material which has been subject to a decarboxylation step in order to convert cannabinoid acids to the corresponding free cannabinoids.

The starting material for the purification

process is an extract containing a cannabinoid or cannabinoid acid obtained from a plant material.

In a preferred embodiment the "extract containing a cannabinoid or cannabinoid acid" may be a solvent extract of a plant material. Preferred solvents include non-polar solvents, also alcohols such as ethanol or methanol and liquid carbon dioxide. Preferably the extract is prepared by dissolving plant material in a solvent, removing insoluble material from the resultant solution (preferably by filtration), and removing the solvent from the solution (preferably by rotary evaporation) to form an extract containing a cannabinoid or cannabinoid acid.

Non-polar solvents are particularly preferred for preparing an initial extract from the starting plant material. Any non-polar solvent capable of solubilising cannabinoids or cannabinoid acids may be used. Preferred non-polar solvents include liquid non-polar solvents comprising lower C5-C12, preferably C5 to C8, straight chain or branched chain alkanes. The most preferred non-polar solvent for the preparation of free cannabinoids is hexane.

In embodiments wherein the method is to be used for the isolation of cannabinoid acids then it is preferred to use an acidified solvent to prepare the initial extract. The primary purpose of this acidification is to prevent/minimise ionisation of the cannabinoid acid, which could otherwise adversely affect the purification process. It is preferred to use acidified non-polar solvents, of the types described above. Acidification may be achieved by the additional of a small volume of acid to the solvent. Generally it is sufficient to add a relatively weak acid, such as acetic acid. For any given purification

process the optimal amount and type of acid used may be determined empirically. A preferred acidified solvent is 0.1% acetic acid in hexane. This is the solvent of choice for preparing an initial extract from the starting plant material in the preparation of cannabinoid acids.

In embodiments of the method where it is desired to purify free cannabinoids, rather than the cannabinoid acids, the plant material may be subjected to a decarboxylation step prior to step (i). The purpose of the decarboxylation step is to convert cannabinoid acids present in the plant material to the corresponding free cannabinoids. Decarboxylation is preferably carried out by heating the plant material to a defined temperature for a suitable length of time. Decarboxylation of cannabinoid acids is a function of time and temperature, thus at higher temperatures a shorter period of time will be taken for complete decarboxylation of a given amount of cannabinoid acid. In selecting appropriate conditions for decarboxylation consideration must, however, be given to minimising thermal degradation of the desirable, pharmacological cannabinoids into undesirable degradation products, particularly thermal degradation of Δ^9 THC to cannabinol (CBN).

Preferably, decarboxylation is carried out in a multi-step heating process in which the plant material is:

i) heated to a first temperature for a first (relatively short) time period to evaporate off retained water and allow for uniform heating of the plant material; and

ii) the temperature is increased to a second temperature for a second time period (typically longer

than the first time period) until at least 95% conversion of the acid cannabinoids to their neutral form has occurred.

Preferably the first step is conducted at a temperature in the range of 100°C to 110°C for 10-20min. More preferably the first temperature is about 105°C and the first time period is about 15 minutes.

Optimum times and temperatures for the second step may vary depending on the nature of the plant material, and more particularly on the cannabinoid which it is intended to isolate from the plant material, and may be easily determined by routine experiment. Suitable conditions may include, for example, a temperature in the range from 115°C to 125°C for a time period in the range from 45 to 75 minutes (typically 120°C for 60 minutes), or a temperature in the range from 135°C to 145°C, for a time period in the range from 15 to 45 minutes.

If the plant material is derived from cannabis plants having a high THC content (defined as >90% THC as a percentage of total cannabinoid content), the second temperature is preferably in the range of 115°C to 125°C, typically 120°C, and the second time period is preferably in the range of 45 minutes to 75 minutes, typically about 60 minutes. More preferably the second temperature is in the range of 100°C to 110°C, typically 105°C, and the second time period is in the range of 60 to 120 minutes. In another embodiment, most preferred for a mass of plant material greater than 4kg, the second temperature is in the range of 140°C to 150°C, preferably 145°C, and the second time period is in the range of 45 to 55 minutes.

Where the starting "plant material" is freshly harvested or "wet" plant material is may be subjected to a drying step to remove excess moisture prior to step (i). For convenience, decarboxylation and drying may be combined in a single heating step or in a multi-step heating process, as described above.

In a particular embodiment of the method of the invention the "extract containing a cannabinoid or cannabinoid acid" prepared from the starting plant material may be a "botanical drug substance" prepared from the plant material, or an ethanolic solution of such a botantical drug substance. In the context of this application a "botanical drug substance" is an extract derived from plant material, which extract fulfils the definition of "botanical drug substance" provided in the Guidance for Industry Botanical Drug Products Draft Guidance, August 2000, US Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research of: "A drug substance derived from one or more plants, algae, or macroscopic fungi. It is prepared from botanical raw materials by one or more of the following processes: pulverisation, decoction, expression, aqueous extraction, ethanolic extraction, or other similar processes."

"Botanical drug substances" derived from cannabis plants include primary extracts prepared by such processes as, for example, maceration, percolation, and solvent extraction. Solvent extraction may be carried out using essentially any solvent that dissolves cannabinoids/cannabinoid acids, such as for example C1 to C5 alcohols (e.g. ethanol, methanol), C5-C12 alkanes (e.g. hexane), Norflurane (HFA134a), HFA227 and carbon dioxide. When solvents such as those listed above are used, the resultant extract

typically contains non-specific lipid-soluble material. This can be removed by a variety of processes including "winterisation", which involves chilling to -20°C followed by filtration to remove waxy ballast, extraction with liquid carbon dioxide and by distillation. General protocols for the preparation of botanical drug substances from cannabis plant material are described in the applicant's published International patent application WO 02/064109.

The botanical drug substance is preferably obtained by carbon dioxide (CO_2) extraction followed by a secondary extraction, e.g. an ethanolic precipitation, to remove a substantial proportion of non-cannabinoid materials, e.g. waxes, wax esters and glycerides, unsaturated fatty acid residues, terpenes, carotenes, and flavenoids and other ballast. Most preferably the botanical drug substance is produced by a process comprising extraction with liquid CO_2, under sub-critical or super-critical conditions, and then a further extraction, preferably an ethanolic precipitation, to remove significant amounts of ballast.

If it is intended to prepare free cannabinoids from the cannabis plant material then the material is preferably heated to a defined temperature for a defined period of time in order to decarboxylate cannabinoid acids to free cannabinoids prior to extraction of the botanical drug substance.

In the most preferred embodiment the botanical drug substance is prepared according to a process comprising the following steps:

i) optional decarboxylation of the plant material,

ii) extraction with liquid CO_2 (most preferably under

sub-critical conditions), to produce a crude botanical drug substance,
iii) precipitation with C1-C5 alcohol to reduce the proportion of non-target materials,
iv) removal of the precipitate (preferably by filtration),
v) optional treatment with activated charcoal, and
vi) evaporation to remove C1-C5 alcohol and water, thereby producing a final botanical drug substance.

A detailed example of such a process is described in the accompanying Examples.

The "extract containing a cannabinoid or cannabinoid acid" is subjected to a chromatographic purification step to produce a partially purified extract. The purpose of this step is to reduce the amount of "non-target", i.e. non-cannabinoid or non-cannabinoid acid material, in the extract and also to provide a degree of separation/fractionation of the various cannabinoid/cannabinoid acid components of the crude plant extract obtained in step (i). Typically, the product of the chromatographic step is collected in multiple fractions, which may then be tested for the presence of the desired cannabinoid/cannabinoid acid using any suitable analytical technique (e.g. TLC). Fractions enriched in the desired cannabinoid/cannabinoid acid may then be selected for further purification.

The chromatographic step will preferably comprise column chromatography, and is preferably based on molecular sizing and polarity. Preferred column matrix materials are hydrophilic lipophilic materials, for example hydroxypropylated cross-linked dextrans such as Sephadex LH-20™. Various different solvents may be used in combination with this type of matrix,

for example dimethyl sulphoxide, pyridine, water, dimethylformamide, methanol, saline, ethylene dichloride, chloroform, propanol, ethanol, isobutanol, formamide, methylene dichloride, butanol, isopropanol, tetrahydrofuran, dioxane, chloroform/dichloromethane etc.

In the most preferred embodiment the chromatographic step comprises column chromatography on a Sephadex LH-20™ column, preferably eluting with a 2:1 mixture of chloroform/dichloromethane. However, any suitable combination of column packing material and solvent having separation characteristics suitable for use in separation (fractionation) of cannabinoids and cannabinoid acids may be used with equivalent effect. The column eluate is typically collected in several fractions. The fractions are tested for the presence of the desired cannabinoid/cannabinoid acid using a suitable analytical technique, and those fractions containing the highest amounts of the desired cannabinoid or cannabinoid acid selected for further processing. Solvent is then removed from the selected fractions, preferably by rotary evaporation.

The partially purified product obtained from the chromatographic step is re-dissolved in a first solvent. Any insoluble residues (e.g. particulate material) are removed from the resultant solution, typically by filtration. The first solvent is then removed, preferably by rotary evaporation. The product of this step is re-dissolved in a second solvent. Again, any insoluble residues (e.g. particulate material) are removed from the resultant solution, typically by filtration. The second solvent is then removed, preferably by rotary evaporation, to produce the final product, which is a substantial pure cannabinoid or cannabinoid acid or a product enriched

in a given cannabinoid or cannabinoid acid.

The purpose of these two "solvent treatment" steps is to remove contaminants, leaving a substantially pure preparation of the desired cannbinoid or cannabinoid acid.

In the preferred embodiment the first and second solvents are different. One of the first or second solvents is a solvent which is substantially more polar than the cannabinoid/cannabinoid acid which it is desired to purify. Treatment with this solvent has the effect of removing unwanted components that are less polar than the desired cannabinoid/cannabinoid acid. The other solvent is a solvent which is substantially less polar than the cannabinoid/cannabinoid acid which it is desired to purify. Treatment with this solvent has the effect of removing unwanted components that are more polar than the desired cannabinoid/cannabinoid acid. The combined effect of sequential treatment with two such solvents is of "topping and tailing" the partially purified extract to yield a substantially pure product. The two solvent treatment steps may be performed in either order. It is immaterial to the overall purification whether the "less polar" or "more polar" contaminants are removed first.

The first and second solvents may be essentially any solvents that dissolve cannabinoids and/or cannabinoid acids and which have the desired polarity in comparison to the cannabinoid/cannabinoid acid which it is desired to isolate.

Preferred solvents for use in these treatment steps include alcohols, particularly C1-C5 alcohols, with methanol being particularly preferred, and also

C5-C12 straight or branched chain alkanes, most preferably pentane. A particularly preferred combination of first and second solvents, which is suitable for use in the preparation of the majority of cannabinoids and cannabinoid acids, is methanol and pentane. These solvents may be used in either order.

The process of the invention generally results in the isolation of substantially pure cannabinoids or cannabinoid acids of high chromatographic purity. Substantially pure cannabinoids or cannabinoid acids are often obtained as crystalline solids, with the exception of Δ^9 THC, which is generally obtained as a colourless low melting point solid.

The process of the invention may be used to prepare substantially pure forms, or products enriched in, essentially any cannabinoids or cannabinoid acids which occur naturally in plant material (including free cannabinoid forms of naturally occurring cannabinoid acids).

The essential features of the process are the same for purification of all cannabinoids and cannabinoid acids. Cannabis plants generally contain complex mixtures of cannabinoid acids and cannabinoids, although depending on the variety of cannabis one type of cannabinoid may pre-dominate. The purpose of the chromatographic step (ii) is to separate the various cannabinoid/cannabinoid acid components of the crude plant extract obtained in step (i). Typically, the product of the chromatographic step is collected in multiple fractions, which may then be tested for the presence of the desired cannabinoid/cannabinoid acid using any suitable analytical technique (e.g. TLC). Fractions enriched in the desired cannabinoid/cannabinoid acid may then

be selected for further purification. Hence, the same simple process steps may be adapted for purification of essentially any plant-derived cannabinoid or cannabinoid acid.

Selectivity for different cannabinoids or cannabinoid acids may be enhanced by selection of appropriate starting plant material. By way of example, if it is desired to prepare substantially pure Δ^9 THC or Δ^9 THCA then "high THC" cannabis plants should preferably be selected as the starting material. Whereas, if it is desired to prepare substantially pure CBD or CBDA then "high CBD" cannabis plants should preferably be selected as the starting material. However, it is to be understood that the process of the invention is of general utility and is not limited to the use of particular cannabis varieties as the starting material.

Working with Cannabis plants and cannabinoids may require a Government licence in some territories but Governments generally make such licences available to parties who apply for the purposes of medicinal research and commercial development of medicines. In the United Kingdom a licence may be obtained from the Home Office.

The precise cannabinoid content of any particular cannabis plant material may be qualitatively and quantitatively determined using analytical techniques well known to those skilled in the art, such as thin-layer chromatography (TLC) or high performance liquid chromatography (HPLC). Thus, one may screen a range of cannabis plants and select those having a high content of the desired cannabinoid acid or cannabinoid for use as starting material in the process of the invention.

With the use of conventional selective breeding techniques it is possible to develop cannabis varieties (chemovars) having varying cannabinoid content. Using such traditional selective breeding techniques the inventors have been able to select cannabis varieties (chemovars) having relatively high content of CBD, or of the minor cannabinoids Δ^9 tetrahydrocannabivarin (Δ^9 THCV), cannabigerol (CBG) or cannabichromene (CBC). General protocols for growing of medicinal cannabis and for testing the cannabinoid content of cannabis plants are described in the applicant's published International patent application WO 02/064109.

Where it is desired to purify free cannabinoids, rather than the corresponding cannabinoid acids, then the process will generally include a "decarboxylation" step to decarboxylate free cannabinoid acids to the corresponding free cannabinoid. As aforesaid, a decarboxylation step may be included prior to step (i) if it is desired to isolate free cannabinoids, or omitted if it is desired to isolate cannabinoid acids.

The process of the invention is particularly preferred for use in the preparation of substantially pure Δ^9 tetrahydrocannabinolic acid (Δ^9 THCA), cannabidiolic acid (CBDA), Δ^9 tetrahydrocannabinol (Δ^9 THC) and Δ^9 tetrahydrocannabivarin (Δ^9 THCV) from cannabis plant material, and in the preparation of extracts of cannabis plant material highly enriched in cannabigerol (CBG) or cannabichromene (CBC).

The invention further relates to substantially pure preparations of certain cannabinoids and cannabinoids and to products highly enriched in certain cannabinoids.

In particular, the invention provides a substantially pure preparation of Δ^9 tetrahydrocannabinolic acid (Δ^9 THCA) having a chromatographic purity of greater than 95%, more preferably greater than 96%, more preferably greater than 97% and most preferably greater than 98% by area normalisation of an HPLC profile. The preparation is typically a pale yellow crystalline solid at room temperature, having a melting point of ~70°C.

The preparation preferably comprises:
less than 2%, preferably less than 1.5%, most preferably 1% or less Δ^9 THC (w/w),

less than 2%, more preferably less than 1.5%, more preferably less than 1% and most preferably less than 0.5% CBD (w/w),

less than 2%, more preferably less than 1.5%, and most preferably less than 1% CBN (w/w).

The inventors are the first to isolate Δ^9 THCA from plant material at this level of purity in crystalline form. Pure Δ^9 THCA is useful as a starting material for the preparation of pure Δ^9 THC by decarboxylation, also as a chromatographic standard.

The preferred method for preparation of substantially pure Δ^9 THCA from cannabis plant material comprises:

i) preparing an extract of the cannabis plant material with 0.1% v/v acetic acid in hexane,
ii) filtering the resultant extract and removing solvent from filtrate by rotary evaporation to form an extract enriched in Δ^9 THCA,

iii) passing a solution of the resulting Δ^9 THCA enriched extract through a column packed with Sephadex-LH20™, eluting with 2:1 chloroform/dichloromethane,

iv) collecting Δ^9 THCA rich fractions eluted from the column and removing solvent by rotary evaporation,

v) re-dissolving the crude Δ^9 THCA obtained in step iv) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,

vi) re-dissolving the product of step v) in pentane, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation to produce Δ^9 THCA crystals.

The cannabis plant material will preferably be derived from cannabis plants having a relatively high Δ^9 THCA content, most preferably cannabis plants containing >90% Δ^9 THCA as a percentage of total cannabinoid content.

The invention further provides a substantially pure preparation of cannabidiolic acid (CBDA) having a chromatographic purity of greater than 90%, more preferable greater than 92% and most preferably greater than 94% by area normalisation of an HPLC profile. The preparation is typically a pale yellow crystalline solid at room temperature, having a melting point in the range of from 45-48°C.

The preparation preferably comprises:

5% or less, preferably 4.5% or less, more preferably 4% or less, more preferably 3.5% or less and most preferably 3% or less CBD (w/w),

less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than

0.4%, more preferably less than 0.2% and most preferably less than 0.1% Δ^9 THCA (w/w),

less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% Δ^9 THC (w/w)

Again, the inventors are the first to isolate CBDA from plant material at this level of purity in crystalline form. Pure CBDA is useful as a starting material for the preparation of pure CBD by decarboxylation, also as a chromatographic standard and may also have pharmaceutical potential. The ability to prepare CBDA at a high level of purity will permit further studies of the pharmaceutical utility of this cannabinoid acid.

The preferred method for preparation of substantially pure CBDA from cannabis plant material comprises:

i) preparing an extract of the cannabis plant material with 0.1% v/v acetic acid in hexane,
ii) filtering the resultant extract and removing solvent from filtrate by rotary evaporation to form an extract enriched in CBDA,
iii) passing a solution of the resulting CBDA enriched extract through a column packed with Sephadex-LH20™, eluting with 2:1 chloroform/dichloromethane,
iv) collecting CBDA rich fractions eluted from the column and removing solvent by rotary evaporation,
v) re-dissolving the crude CBDA obtained in step iv) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vi) re-dissolving the product of step v) in pentane,

removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation to produce CBDA crystals.

The cannabis plant material will preferably be derived from cannabis plants having a relatively high CBDA content, most preferably cannabis plants containing >90% CBDA as a percentage of total cannabinoid content.

The invention further provides a substantially pure preparation of Δ^9 tetrahydrocannabinol (Δ^9 THC) having a chromatographic purity of greater than 99% by area normalisation of an HPLC profile. The preparation is a semi-solid at room temperature.

The preparation preferably comprises less 0.5%, preferably than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBD (w/w),

less than 0.5%, preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBN (w/w).

Most preferably the preparation contains no detectable (<0.1%) CBD and no detectable CBN (<0.1%), as analysed by HPLC.

The inventors are the first to isolate Δ^9 THC from plant material at >99% purity and in semi-solid form. Δ^9 THC has previously been reported in the literature as a yellow oil and has never been obtained in crystalline form. The pure Δ^9 THC is of obvious utility as an active pharmaceutical agent, and is also useful as a chromatographic standard, particularly as a comparative standard in the qualitative analysis of botanical drug substances derived from cannabis. The

availability of highly pure Δ^9 THC will also facilitate studies of the pharmacology of Δ^9 THC.

The preferred method for preparation of substantially pure Δ^9 THC comprises:

i) obtaining an ethanolic solution of a botanical drug substance from decarboxylated cannabis plant material,
ii) passing the solution obtained in step i) through a column of activated charcoal, and collecting the eluate,
iii) remove solvent from the eluate by rotary evaporation to give a Δ^9 THC enriched fraction,
iv) passing a solution of the resulting Δ^9 THC enriched extract through a column packed with Sephadex LH20, eluting with 2:1 chloroform/dichloromethane,
v) collecting Δ^9 THC rich fractions and removing solvent by rotary evaporation,
vi) re-dissolving the crude Δ^9 THC prepared in step v) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vii) re-dissolving the crude Δ^9 THC prepared in step vi) in pentane, removing insoluble residue by filtration and removing solvent from the filtrate by rotary evaporation to give a semi-solid preparation of Δ^9 THC.

In this method the ethanolic solution of a botanical drug substance from decarboxylated cannabis plant material is preferably obtained by a method comprising the following steps:

i) harvesting cannabis plant material,
ii) decarboxylation of the plant material,
iii) extraction with liquid carbon dioxide (CO_2), removal of CO_2 to recover crude extract,

iv) dissolution of crude extract in ethanol followed by chilling of the solution to precipitate unwanted waxes,

v) removal of unwanted waxy material by cold filtration.

The (decarboxylated) cannabis plant material will preferably be derived from cannabis plants having a relatively high THC content, most preferably cannabis plants containing >90% THC (Δ^9 THCA plus Δ^9 THC) as a percentage of total cannabinoid content. The plant material is subject to decarboxylation in order to convert the naturally occurring Δ^9 THCA into Δ^9 THC.

The invention still further relates to a substantially pure preparation of Δ^9 tetrahydrocannabivarin (Δ^9 THCV) having a chromatographic purity of greater than 95%, more preferable greater than 96%, more preferable greater than 97%, more preferable greater than 98%, and most preferable greater than 99% by area normalisation of an HPLC profile. The preparation is typically a crystalline solid at room temperature.

The preparation preferably comprises less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBD (w/w),

less than 2.0%, preferably less than 1.5%, more preferably less than 1.0% and most preferably 0.5% or less Δ^9 THC (w/w),

less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most

preferably less than 0.1% CBN (w/w).

Again the inventors are the first to isolate Δ^9 THCV from plant material at this level of purity and in crystalline form. The availability of pure Δ^9 THCV will permit studies of the pharmacology of this minor cannabinoid and evaluation of its pharmaceutical potential. Pure Δ^9 THCV is also useful as a chromatographic standard and as a starting material for the preparation of pure cannabivarin (CBV), for example by thermal degradation of Δ^9 THCV in air.

The preferred method for preparation of substantially pure Δ^9 THCV from plant material comprises:

i) obtaining an ethanolic solution of a botanical drug substance from cannabis plant material,
ii) passing the solution obtained in step i) through a column of activated charcoal, and collecting the eluate,
iii) remove solvent from the eluate by rotary evaporation to give a Δ^9 THCV enriched fraction,
iv) passing a solution of the resulting Δ^9 THCV enriched extract through a column packed with Sephadex LH20, eluting with 2:1 chloroform/dichloromethane,
v) collecting Δ^9 THCV rich fractions and removing solvent by rotary evaporation,
vi) re-dissolving the crude Δ^9 THCV prepared in step v) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vii) re-dissolving the crude Δ^9 THCV prepared in step vi) in pentane, removing insoluble residue by filtration and removing solvent from the filtrate by rotary evaporation to give crystals of Δ^9 THCV.

The ethanolic solution of a botanical drug substance from cannabis plant material is preferably obtained by a method comprising the following steps:

i) harvesting and decarboxylating cannabis plant material,
ii) extraction with liquid carbon dioxide (CO_2), removal of CO_2 to recover crude extract,
iii) dissolution of crude extract in ethanol followed by chilling of the solution to precipitate unwanted waxes,
iv) removal of unwanted waxy material by cold filtration.

The cannabis plant material will preferably be derived from cannabis plants having a relatively high Δ^9 THCV content.

The invention still further comprises a product enriched in cannabigerol (CBG) having a chromatographic purity of greater than 90%, preferably greater than 92% by area normalisation of an HPLC profile.

The product preferably comprises less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBD (w/w),

less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably 0.1% or less Δ^9 THC (w/w).

The product most preferably contains no detectable (<0.1%) CBN or CBD and no more than 0.1% Δ^9

THC, as analysed by HPLC.

Again, the inventors are the first to prepare cannabis plant extracts containing the minor cannabinoid CBG at this level of chromatographic purity. The availability of such enriched extracts will permit further evaluation of the pharmacology of CBG in order to assess its pharmaceutical potential. The enriched extract is also useful as a reference standard in chromatographic characterisation of cannabis-derived medicines.

The preferred method of preparing enriched CBG extracts from cannabis plant material comprises:
i) decarboxylating the cannabis plant material,
ii) preparing an extract of the decarboxylated cannabis plant material with hexane,
iii) filtering the resultant extract and removing solvent from filtrate by rotary evaporation to form an extract enriched in CBG,
iv) passing a solution of the resulting CBG enriched extract through a column packed with Sephadex-LH20™, eluting with 2:1 chloroform/dichloromethane,
v) collecting CBG rich fractions eluted from the column and removing solvent by rotary evaporation,
vi) re-dissolving the crude CBG obtained in step v) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vii) re-dissolving the product of step vi) in pentane, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation to produce a highly enriched CBG extract.

The cannabis plant material will preferably be derived from cannabis plants having a relatively high CBG content.

The invention still further comprises a product enriched in cannabichromene (CBC) having a chromatographic purity of greater than 80%, more preferably greater than 85% by area normalisation of an HPLC profile.

The product preferably comprises less than 5%, preferably less than 4%, more preferably less than 3%, more preferably less than 2% and most preferably 1% or less CBD (w/w),

less than 2%, preferably less than 1.5%, more preferably less than 1.0%, more preferably less than 0.5% and most preferably 0.3% or less Δ^9 THC (w/w),

less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably 0.1% or less CBN (w/w).

Again, the inventors are the first to prepare cannabis plant extracts containing the minor cannabinoid CBC at this level of chromatographic purity. The availability of such enriched extracts will permit further evaluation of the pharmacology of CBC in order to assess its pharmaceutical potential. The enriched extract is also useful as a reference standard in chromatographic characterisation of cannabis-derived medicines.

The preferred method for preparing enriched CBC extracts from cannabis plant material comprises:
i) decarboxylating the cannabis plant material,
ii) preparing an extract of the decarboxylated cannabis plant material with hexane,
iii) filtering the resultant extract and removing solvent from filtrate by rotary evaporation to form an

extract enriched in CBC,
iv) passing a solution of the resulting CBC enriched extract through a column packed with Sephadex-LH20™, eluting with 2:1 chloroform/dichloromethane,
v) collecting CBC rich fractions eluted from the column and removing solvent by rotary evaporation,
vi) re-dissolving the crude CBC obtained in step v) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vii) re-dissolving the product of step vi) in pentane, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation to produce a highly enriched CBC extract.

The cannabis plant material will preferably be derived from cannabis plants having a relatively high CBC content.

The invention will be further understood with reference to the following experimental examples, together with the accompanying Figures, in which:

Figure 1 shows TLC a profile of crystalline Δ^9 THCA, compared to starting material (from G1 cannabis chemovar) and CBD and Δ^9 THC standards.

Figure 2 shows HPLC profiles of purified Δ^9 THCA, compared to starting material (from G1 cannabis chemovar).

Figure 3 shows TLC profile of crystalline CBDA, compared to starting material (from G5 cannabis chemovar) and CBD and Δ^9 THC standards.

Figure 4 shows HPLC profiles of crystalline CBDA, compared to starting material (from G5 cannabis

chemovar).

Figure 5 shows TLC profiles of purified Δ^9 THC compared to BDS starting material and CBD and Δ^9 THC standards.

Figure 6 shows HPLC profiles of purified Δ^9 THC compared to starting material (BDS).

Figure 7 shows comparative HPLC profiles of purified Δ^9 THC and commercially available Δ^9 THC standard (Sigma).

Figure 8 shows GC profiles of purified Δ^9 THC and starting material (BDS).

Figure 9 shows TLC profiles of purified Δ^9 THCV and THCV starting material (BDS) compared to CBD and Δ^9 THC standards.

Figure 10 shows HPLC profiles of Δ^9 THCV and starting material (BDS).

Figure 11 shows GC profiles of purified Δ^9 THCV and starting material (BDS).

Figure 12 shows TLC profiles of enriched CBG extract and starting material (BDS from G41 chemovar-decarboxylated) compared to CBD and Δ^9 THC standards.

Figure 13 shows HPLC profiles of enriched CBG extract and starting material (BDS from G41 chemovar-decarboxylated).

Figure 14 shows GC profiles of enriched CBG extract and starting material (BDS from G41 chemovar-decarboxylated).

Figure 15 shows TLC profiles of enriched CBC extract and starting material (BDS from G80 chemovar-decarboxylated) compared to CBD and Δ^9 THC standards.

Figure 16 shows HPLC profiles of enriched CBC extract and starting material (BDS from G80 chemovar-decarboxylated).

Figure 17 shows GC profiles of enriched CBC extract and starting material (BDS from G80 chemovar-decarboxylated).

Examples

Materials and methods

Plant material

GW Pharma Ltd has developed distinct varieties of Cannabis plant hybrids to maximise the output of the specific chemical constituents, cannabinoids. Various types of plant are used; one chemovar, designated G1 or "high THC" chemovar, produces >90% total cannabinoid content as Δ^9 THC (naturally occurring in the plant in the form of Δ^9 THCA) and a further chemovar, designated G5 or "high CBD" chemovar produces >90% total cannabinoid content as CBD (naturally occurring in the plant in the form of CBDA). Other chemovars yield significant amounts of the minor cannabinoids Δ^9 THCV (G9 chemovar), CBG (G41 chemovar) and CBC (G80 chemovar). Alternative varieties can be obtained - see for example, Common cannabinoids phenotypes in 350 stocks of cannabis, Small and Beckstead, Lloydia vol 36b , 1973 p144-156 - and bred using techniques well known to the skilled man to maximise cannabinoid content.

Solvents

All solvents used in the isolation and analysis of the cannabinoids; n-pentane, hexane, chloroform, dichloromethane, di-ethyl ether, acetonitrile, water, methanol and glacial acetic acid were, unless otherwise stated, of chromatographic or A.R. grade.

Standards

Reference materials from Sigma were used as standards in the analysis of extracts, intermediates and finished products, these were: Δ^9 THC in methanol BN 10601/B (ca. 1 mg/ml) and CBD in methanol BN 10601/C (ca. 1 mg/ml).

Solvent extraction step

For preparation of Δ^9 THCA and CBDA samples of G1, THC cannabis chemovar (100 g) and G5, CBD cannabis chemovar (100 g) were extracted twice with 0.1 % v/v glacial acetic acid in hexane (A.R. grade) at a solvent:herb ratio of 15:1. The resulting extracts were filtered and then solvent removed by rotary evaporation to yield crude extracts enriched in the respective cannabinoid acids and suitable for further processing.

For preparation of cannabigerol (CBG) and cannabichromene (CBC) samples of G41, CBG cannabis chemovar (100 g) and G80, CBC cannabis chemovar (100 g) were decarboxylated at 120°C for 1 hour and then extracted twice with hexane at a solvent:herb ratio of 15:1. Following the removal of solvent, this yielded a crude extract enriched in the respective compounds CBG and CBC and suitable for further processing.

For preparation of Δ^9 THC and Δ^9 THCV ethanolic solutions of botanical drug substances were prepared, respectively, from high THC and high THCV cannabis

chemovars according to the following process:

harvest cannabis plant material, dry, reduce particle size by milling to less than 2000 µm

↓

for Δ^9 THC decarboxylate milled plant material by heating to approximately 105°C for 15 minutes, followed by approximately 145°C for minimum of 55 minutes (NB decarboxylation conditions may be varied depending on nature of target cannabinoid)

↓

extract with liquid carbon dioxide (CO_2) [Food Grade] for up to 10 hours Conditions: Approximately 60 bar ± 10 bar pressure and 10°C ± 5°C

↓

Removal of CO_2 by depressurisation to recover crude extract

↓

"Winterisation"-Dissolution of crude extract in ethanol followed by chilling solution (-20°C ± 5°C/up to 52hours) to precipitate unwanted waxes

↓

Removal of unwanted waxy material by cold filtration (20mm filter)

↓

ethanolic solution of BDS
(Stored at -20°C ± 5°C)

Extraction using liquid CO_2 is carried out under sub-critical conditions at a temperature of approximately 10°C ±5°C using a pressure of approximately 60 bar ±10bar. Decarboxylated plant material is packed into a single column and exposed to liquid CO_2 under pressure for approximately 8 hours, CO_2 mass flow 1250kg/hr ±20%.

Following depressurisation and venting off of the CO_2 the crude BDS extract is collected into sealed vessels. The crude BDS extract is held at -20°C ± 5°C.

The crude BDS extract contains waxes and long chain molecules. Removal is by "winterisation", whereby the crude BDS extract is warmed to e.g. 40°C ± 4°C to liquefy the material. Ethanol is added in the ratio of 2:1 ethanol volume to weight of crude BDS extract. The ethanolic solution is then cooled to -20°C ± 5°C and held at this temperature for approximately 48 hours.

On completion of the winterisation the precipitate is removed by cold filtration through a 20µm filter, to give an ethanolic solution of the BDS.

Preliminary charcoal clean-up may be carried out by passing the ethanolic BDS solution (500 mg/ml) through a disposable plastic column (130 mm x 27 mm i.d) packed with activated charcoal (decolourcarb DCL GDC grade, from Sutcliffe Speakman Carbons, 15.4 g per unit). Absolute ethanol B.P. (Hayman) is used as the solvent.

Ethanol and any water that may be present are removed by evaporation, e.g. rotary evaporation or thin film evaporation under reduced pressure(60°C ± 2°C, with vapour at 40°C ± 2°C /172 mbar and 72 mbar±4mbar). The resulting product may be applied directly to the chromatography column.

Column chromatography step

Low pressure column chromatography separations were carried out using a glass column (length x internal diameter = 1560 mm x 24 mm), packed with

Sephadex LH-20™ (Fluka). The column length:internal diameter ratio was therefore 65:1. A 2:1 chloroform/dichloromethane mixture was used as eluant. Eluate was collected as 50 ml fractions.

For purification of Δ^9 THCA and CBDA approximately 20 ml of crude extract containing the equivalent of 100 g herb was applied to a glass column (dimensions: length 1560 mm x internal diameter 24 mm), packed with 400 g of Sephadex LH-20™ stationary phase, as described above. The qualitative composition of eluted fractions was monitored by TLC.

For the purification of Δ^9 THC, 2.5 g of charcoal purified BDS (THC) extract was processed through the above low pressure chromatography system, (i.e. stationary phase:sample ratio of 160:1). Eluted fractions were analysed for Δ^9 THC content by TLC.

For purification of CBG and CBC approximately 20 ml of crude extract containing the equivalent of 100 g herb was applied to a glass column (dimensions: length 1560 mm x internal diameter 24 mm), packed with 400 g of sephadex stationary phase.

For the purification of Δ^9 THCV, 3 g of charcoal purified BDS (THCV) extract was processed through the above low pressure chromatography system, (i.e. stationary phase:sample ratio of 133:1).

<u>Solvent treatment steps</u>

Steps of re-dissolving extracts in the first and second solvents, filtering to remove insoluble material and removing solvent by rotary evaporation are carried out according to standard laboratory procedures, such as would be known to those skilled in the art.

TLC analysis

The qualitative composition of fractions eluted from the chromatography column and other intermediates was monitored by TLC.

TLC uses both retention time and characteristic spot colour to effectively identify the cannabinoid/cannabinoid acid components in a complex mixture. Methanolic solutions of the fractions eluted from the chromatographic column are prepared for TLC analysis. An aliquot is spotted onto a TLC plate, alongside suitable reference samples (e.g. for at least Δ^9 THC and CBD). Following exposure to Fast Blue B reagent, THC and THCA present as pink spots, while CBD and CBDA are orange in colour. Neutrals can be distinguished from the acids by comparison of the Rf value to that obtained for the standards. Identity is confirmed by comparison of Rf and colour of the sample spot, to that obtained for the appropriate standard.

A typical TLC protocol is as follows:

a) Materials and methods

Equipment:
Application device capable of delivering an accurately controlled volume of solution i.e 1 μl capillary pipette or micro litre syringe.

TLC development tank with lid

Hot air blower

Silica gel G TLC plates (SIL N-HR/UV254), 200 μm layer with fluorescent indicator on polyester support.

Dipping tank for visualisation reagent.

Mobile phase 80% petroleum ether 60:80/20% Diethyl ether.

Visualisation reagent 0.1% w/v aqueous Fast Blue B salt BN (Sigma Corp) (100mg in 100ml de-ionised water). An optional method is to scan at UV 254 and 365 nm.

b) Sample preparation

i) Herbal raw material

Approximately 200mg of finely ground, dried cannabis is weighed into a 10ml volumetric flask. Make up to volume using methanol:chloroform (9:1) extraction solvent.

Extract by ultrasound for 15 minutes. Decant supernatant and use directly for chromatography.

ii) Eluted column fractions and intermediate extracts are dissolved in methanol then used directly. Suitable dilutions may be determined empirically.

iii) Final products

The final products (pure cannabinoids or enriched extracts) are dissolved in methanol to s suitable concentration (which may be determined empirically) then used directly for chromatography. All sample preparations should produce a final concentration of about 0.5 mg/ml.

iv) Botanical drug substance

Accurately weigh approximately 50 mg of botanical drug substance into a 25 ml volumetric flask. Dissolve to make volume with HPLC grade methanol.

c) Standards

0.1 mg/ml Δ^9-THC in methanol (Sigma).
0.1mg/ml CBD in methanol (Sigma).

The standard solutions are stored frozen at -20°C between uses and are used for up to 12 months after initial preparation.

d) Test solutions and method

Apply to points separated by a minimum of 10mm.

i) either 5 μl of herb extract or 1 μl of pure cannabinoid/enriched extract solution or 1 μl of diluted column eluate as appropriate,
ii) 5 μl of 0.1 mg/ml Δ^9-THC in methanol standard solution,
iii) 5 μl of 0.1mg/ml CBD in methanol standard solution.

Dry the prepared plate with a hot air blower.

Place the base of the TLC plate in a development tank containing the mobile phase and saturated with vapour.

Elute the TLC plate through a distance of 8cm, then remove the plate. Allow solvent to evaporate from the plate and then repeat the elution for a second time (double development). Remove plate and allow it to dry in air.

The entire plate is briefly immersed in the Fast Blue B reagent until the characteristic red/orange colour of cannabinoids begins to develop. The plate is removed and allowed to dry under ambient conditions in the dark.

Cannabinoids will give an orange-purple colour:

Cannabidiol	CBD	orange (fastest running)
Δ^9 Tetrahydrocannabinol	THC	pink
Cannabinol	CBN	purple
Cannabichromene	CBC	pink purple
Cannabigerol	CBG	orange
Δ^9 tetrahydrocannabivarin	THCV	purple

The corresponding acids form streaks of the same colour as the neutral component spots. The acids run at lower R_f.

HPLC analysis

The composition of the isolated products may be determined by HPLC analysis.

A typical HPLC assay for Δ^9 THC, Δ^9 THCA, CBD, CBDA and CBN may be carried out as follows:

a) Materials and methods

Chromatography Equipment and conditions:

Equipment	Agilent (HP)1100 HPLC system with variable wavelength UV detector or diode array detector.
HPLC Column	Discovery C8 5µm 15cm x 0.46cm
Pre-Column	Kingsorb C18 5µm 3cm x 0.46cm
Mobile Phase	Acetonitrile : Methanol : 0.25% w/v acetic acid (16:7:6 by volume)
Column Temp	25°C
Flow Rate	1.0ml min-1

Detection	220nm 600mA f.s.d. Second wavelength 310nm
Injection Volume	10µl
Run Time	20-25 minutes (may be extended for samples containing small amount of late-eluting peaks)
Elution Order	CBD, CBDA, Δ^9 THCV, CBN, Δ^9 THC, CBC, Δ^9 THCA

b) Sample preparation

Samples of "pure" cannabinoids/cannabinoid acids and enriched extracts are diluted in methanol prior to HPLC analysis. Optimal dilutions may be determined empirically.

Herbal cannabis samples are prepared by taking a 100mg sample and treating this with 5 or 10ml of Methanol/Chloroform (9/1 w/v). The dispersion is sonicated in a sealed tube for 10 minutes, allowed to cool and an aliquot is centrifuged and suitably diluted with methanol prior to chromatography.

c) Standards

Stock standard solutions of CBD, CBN and Δ^9 THC in methanol at approximately 1mg ml-1 are stored at -20°C. Diluted working standards (0.1 mg/ml for Δ^9 THC and CBD and 0.01 mg/ml for CBN) are prepared in methanol from the stock standards and stored at -20°C (maximum period of twelve months after initial preparation). After preparation, standard solutions must be aliquoted into vials to reduce the amount of standard exposed to room temperature. Prior to use in an HPLC sample assay, the required number of standard vials are removed and allowed to equilibrate to room temperature.

Injection of each standard is made in triplicate prior to the injection of any test solution. At suitable intervals during the processing of test solutions, repeat injections of standards are made. In the absence of reliable CBDA and Δ^9 THCA standards, these compounds are analysed using respectively the CBD and Δ^9 THC standard response factors.

d) Test solutions

Diluted test solutions are made up in methanol and should contain analytes in the linear working range of 0.02-0.2 mg/ml.

e) Chromatography Acceptance Criteria:

The following acceptance criteria are applied to the results of each sequence as they have been found to result in adequate resolution of all analytes (including the two most closely eluting analytes CBD and CBDA)

Table 1- Retention time windows and Relative Retention Time (RRT) to Δ^9 THC for each analyte

Cannabinoid	Retention time (minutes)	RRT (THC)
CBD	5.1-5.8	0.58
CBN	7.4-8.3	0.83
Δ^9 THC	9.0-10.0	1.00
CBDA	5.5-6.2	0.615
Δ^9 THCV	5.9-6.2	0.645
CBC	11.6-12.8	1.30
Δ^9 THCA	14.6-16.0	1.605

Table 2- Peak Shape (Symmetry Factor according to British Pharmacopoeia method)

Cannabinoid	Symmetry factor
CBD	<1.30
CBN	<1.25
Δ^9 THC	<1.35

f) Data Processing

Cannabinoids can be subdivided into neutral and acidic- the qualitative identification can be performed using the DAD dual wavelength mode. Acidic cannabinoids absorb strongly in the region of 220nm-310nm. Neutral cannabinoids only absorb strongly in the region of 220nm.

Routinely, only the data recorded at 220 nm is used for quantitative analysis.

The DAD can also be set up to take UV spectral scans of each peak, which can then be stored in a spectral library and used for identification purposes.

Data processing for quantitation utilises batch processing software on the Hewlett Packard Chemstation.

g) calculation:

Chromatographic purity of cannabinoid samples is calculated as a % of total cannabinoid content by area normalization.

<u>Capillary gas chromatography (GC) analysis</u>

a) Chromatography equipment and conditions

Equipment	Agilent (HP) 5890 or 6890 GLC system with HP7673 Autosampler and FID detector
GLC column	SE54(EC5) 30m x 0.32mm i.d. (Alltech) phase thickness 0.25 µm
Flow rate	Constant pressure (10.3 psi). Normal initial flow rate 34cm sec^{-1} (2.0 ml min^{-1})
Column oven	70°C initially then ramp 5°C min^{-1} to 250°C. Hold at 250°C for 15 minutes.
Injector temp	250°C
Detector temp	325°C
Injection Vol	1 µl, split ratio 2.5:1
Run time	45 minutes
Fuel gases	Hydrogen 40 ml min^{-1} Air 450 ml min^{-1} Helium 45 ml min^{-1}

b) Standard preparation

Stock standard solutions of CBD, CBN and Δ^9 THC in methanol at approximately 1mg ml-1 are stored at -20°C. Diluted working standards (0.1 mg/ml for Δ^9 THC and CBD and 0.01 mg/ml for CBN) are prepared in methanol from the stock standards and stored at -20°C (maximum period of twelve months after initial preparation). Allow an aliquot pipetted into an autosampler vial to equilibriate to room temperature prior to use in a GC assay.

c) Sample preparation

Samples of final products, i.e. "pure" cannabinoids/cannabinoid acids and enriched extracts

are diluted in methanol prior to HPLC analysis. Optimal dilutions may be determined empirically.

Cannabis plant material samples are prepared by taking 100mg chopped dried material and treating this with 5 or 10ml of Methanol/Chloroform (9:1 v/v). Extract the sample in an ultrasonic bath for 15 minutes and allow to stand in the dark for 18 hours.

d) Chromatography procedure

Standard solutions are used to provide quantitative and retention time data. These can be typically injected in triplicate prior to the injection of any sample solutions and then singularly at suitable intervals during the run, with a maximum of 10 test samples in between standards.

Table 3-Retention times

THCV	33.7-34.5 minutes
CBD	35.6-36.3 minutes
Δ^9 THC	37.2-38.1 minutes
CBN	38.5-39.1 minutes

Example 1 - Preparation of Δ^9 THCA

Summary of process:

Extract THC herb (G1 chemovar) with 0.1% v/v acetic acid in hexane.

↓

Filter and remove solvent from filtrate on rotary evaporator.

↓

Pass a solution of the resulting THCA enriched extract through a column packed with Sephadex LH20, eluting with 2:1 chloroform/dichloromethane.

↓

Collect THCA rich fractions and remove solvent by rotary evaporation.

↓

Re-dissolve crude THCA in methanol and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Re-dissolve crude THCA in pentane and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Δ^9 THCA crystals

Results:

Yield:
100 g of G1 chemovar yields approx 5 g of purified Δ^9 THCA

Characteristics:
Pale yellow crystalline solid.

Chromatographic purity = 98% by area normalization.

CBD < 0.5% w/w
THC = 1.0% w/w
CBN < 1.0% w/w

Melting point = 70°C (with decomposition).

Material slowly decarboxylates in solution
Δ^9 THCA → Δ^9 THC + CO_2

Example 2-Preparation of CBDA

Summary of process:

Extract CBD herb (G5 chemovar) with 0.1% v/v acetic acid in hexane.

↓

Filter and remove solvent from filtrate on rotary evaporator.

↓

Pass a solution of the resulting CBDA enriched extract through a column packed with Sephadex LH20, eluting with 2:1 chloroform/dichloromethane.

↓

Collect CBDA rich fractions and remove solvent by rotary evaporation.

↓

Re-dissolve crude CBDA in methanol and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Re-dissolve crude CBDA in pentane and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

CBDA crystals

Yield:
100 g of G5 chemovar yields approx 5 g of purified CBDA.

Characteristics:
Pale yellow crystalline solid

Melting Point = 45-48°C

Chromatographic purity = 94 % CBDA by area normalisation

*CBD 3 %.
THCA non detected i.e. < 0.1 %
THC non detected i.e. < 0.1 %

Material slowly decarboxylates in solution
CBDA → CBD + CO_2

* As CBDA does not co-elute with CBD during processing of the extract in the low pressure column chromatography method employed, the detected CBD is likely to be formed from the breakdown of the CBDA during processing and analysis. This undesirable decarboxylation of the purified material might be minimised by manipulation of CBDA at sub-ambient temperatures.

Example 3 - Preparation of Δ^9 THC

Summary of process:

Ethanolic solution of BDS (approx 400 mg/ml) passed through a column of activated charcoal, and eluate collected.

↓

Remove solvent by rotary evaporation to give THC enriched fraction.

↓

Pass a solution of the resulting THC enriched extract through a column packed with Sephadex LH20, eluting with 2:1 chloroform/dichloromethane.

↓

Collect THC rich fractions and remove solvent by rotary evaporation.

↓

Re-dissolve crude THC in methanol and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Re-dissolve crude THC in pentane and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Δ^9 THC SEMI-SOLID

Yield:
3.5 g of Δ^9 THC BDS yields approx 1.5 g of purified Δ^9 THC.

Characteristics:
Clear semi-solid which rapidly takes on a purple colour when exposed to air.
(This colour change is reversible when the material is redissolved in a suitable solvent).

Chromatographic purity > 99% Δ^9 THC by area normalization.

Chromatographic purity superior to commercially available Δ^9 THC Sigma standard

CBD non detected i.e. < 0.1%
CBN non detected i.e. < 0.1%

Identity confirmed by HPLC, GC and TLC retention behaviour compared to Δ^9 THC Sigma standard.

Example 4-Preparation of Δ^9 THCV

Summary of process:

Ethanolic solution of BDS, derived from G9 chemovar, passed through column of activated charcoal, and eluate collected.

↓

Remove solvent by rotary evaporation to give enriched cannabinoid extract.

↓

Pass a solution of the resulting concentrated extract through a column packed with Sephadex LH20 and eluting with 2:1 chloroform/dichloromethane.

↓

Collect THCV rich fractions and remove solvent by rotary evaporation.

↓

Re-dissolve crude THCV enriched fractions in methanol and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Re-dissolve crude THCV enriched fractions in pentane and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Crystalline THCV

Yield:
4.0 g of Δ^9 THCV BDS yields approx 1.3 g of purified Δ^9 THCV.

Characteristics:
Off white crystals which rapidly take on a purple colour when exposed to air. This colour change is reversible when the crystals are redissolved.

Chromatographic purity >99% by area normalization.

CBD non detected i.e. < 0.1 %
THC 0.5 %
CBN non detected i.e. < 0.1 %

Superior to BDS THCV, which contains 75 % THCV & 17 % THC as % of total cannabinoids, for studies of chemistry and pharmacology of THCV.

Identity confirmed by HPLC & GC retention times versus THCV fraction previously authenticated by GC-MS.

Example 5-Preparation of cannabigerol (CBG)

Summary of process:

Extract decarboxylated G41 chemovar with hexane.

↓

Filter and remove solvent from filtrate on rotary evapprator.

↓

Pass a solution of the resulting concentrated extract through a column packed with Sephadex LH20 and eluting with 2:1 chloroform/dichloromethane.

↓

Collect CBG rich fractions and remove solvent by rotary evaporation.

↓

Re-dissolve crude CBG enriched fractions in methanol and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Re-dissolve crude CBG enriched fractions in pentane and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Highly enriched CBG extract

Yield:
100 g of G41 chemovar yields approx 300 mg of CBG enriched fraction.

Characteristics:
Orange/yellow semi-solid.

Identification by GC retention index relative to THC & CBD standards [ref: Brenneisen, R. & El Sohly, M.A., "Chromatographic & spectroscopic Profiles of Cannabis of Different Origins: Part I," Journal of Forensic Sciences, JFSCA, vol.33, No.6, pp.1385-1404, 1988].

Chromatographic purity > 92% by area normalization.

CBD non-detected i.e. < 0.1%
THC 0.1%
CBN non-detected i.e. < 0.1%

Example 5-Preparation of cannabichromene (CBC)

Summary of process:

Extract decarboxylated G80 chemovar with hexane.

↓

Filter and remove solvent from filtrate on rotary evaporator.

↓

Pass a solution of the resulting concentrated extract through a column packed with Sephadex LH20 and eluting with 2:1 chloroform/dichloromethane.

↓

Collect CBC rich fractions and remove solvent by rotary evaporation.

↓

Re-dissolve crude CBC enriched fractions in methanol and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Re-dissolve crude CBC enriched fractions in pentane and remove insoluble residue by filtration.

↓

Remove solvent from filtrate by rotary evaporation.

↓

Highly enriched CBC extract.

Yield:
100 g of G80 chemovar yields approx 300 mg of CBC enriched fraction.

Characteristics:
Orange/yellow semi-solid.

Identification by GC retention index relative to THC & CBD standards [ref: Brenneisen, R. & El Sohly, M.A., "Chromatographic & spectroscopic Profiles of Cannabis of Different Origins: Part I," Journal of Forensic Sciences, JFSCA, vol.33, No.6, pp.1385-1404, 1988].

Chromatographic purity > 85% by area normalization.

CBD 1.0 %
THC 0.3 %
CBN 0.1 %

Claims

1. A method of obtaining a substantially pure cannabinoid or cannabinoid acid or a product enriched in a given cannabinoid or cannabinoid acid from a plant material, comprising:
i) obtaining an extract containing a cannabinoid or cannabinoid acid from a plant material;
ii) subjecting the extract of step (i) to a chromatographic step to produce a partially purified extract;
iii) dissolving the partially purified extract in a first solvent, removing any insoluble material therefrom and removing the solvent; and
iv) dissolving the product obtained in step iii) in a second solvent, removing any insoluble material therefrom, and removing the solvent to obtain the substantially pure cannabinoid or cannabinoid acid or the product enriched in a given cannabinoid or cannabinoid acid.

2. A method according to claim 1 wherein the extract containing a cannabinoid or cannabinoid acid obtained in step (i) is prepared by a process comprising solvent extraction of the plant material.

3. A method according to claim 2 wherein step (i) comprises dissolving the plant material in a solvent, removing any insoluble material from the resultant solution and removing the solvent to form an extract containing a cannabinoid or cannabinoid acid.

4. A method according to claim 2 or claim 3 wherein the solvent is a non-polar solvent, ethanol, methanol or carbon dioxide.

5. A method according to claim 4 wherein the non-polar solvent comprises a straight or branched chain C5-C12 alkane.

6. A method according to claim 5 wherein the non-polar solvent is hexane.

7. A method according to claim 2 or claim 3, wherein the solvent is acidified.

8. A method according to claim 7 wherein the solvent is an acidified non-polar solvent.

9. A method according to claim 8 wherein the solvent is an acidified straight or branched chain C5-C12 alkane.

10. A method according to claim 9 wherein the solvent is 0.1% v/v acetic acid in hexane.

11. A method according to any one of claims 1 to 10, which includes a further step, prior to step (i), of decarboxylating the plant material.

12. A method according to claim 1 wherein the extract containing a cannabinoid or cannabinoid acid obtained in step (i) comprises a botanical drug substance derived from the plant material.

13. A method according to claim 12 wherein the botanical drug substance is prepared by a process comprising solvent extraction of the plant material.

14. A method according to claim 13 wherein the botanical drug substance is prepared by extraction with carbon dioxide.

15. A method according to claim 14 wherein the botanical drug substance is prepared by a process comprising extraction with carbon dioxide (CO_2), followed by a secondary extraction step to remove a proportion of the non-target materials.

16. A method according to claim 15 wherein the secondary extraction step is ethanolic precipitation.

17. A method according to claim 15 or claim 16 wherein the process for preparing the botanical drug substance further includes a charcoal clean-up step.

18. A method according to claim 17 wherein the botanical drug substance is prepared by a process comprising:
i) optional decarboxylation of the plant material,
ii) extraction with liquid CO_2, to produce a crude botanical drug substance,
iii) precipitation with C1-C5 alcohol to reduce the proportion of non-target materials,
iv) removal of the precipitate,
v) treatment with activated charcoal, and
vi) evaporation to remove C1-C5 alcohol and water,
thereby producing a final botanical drug substance.

19. A method according to any one of the preceding claims wherein the chromatographic step comprises column chromatography.

20. A method according to any one of the preceding claims wherein the chromatographic step is based on molecular sizing and polarity.

21. A method according to claim 20 wherein the chromatographic step is carried out using a Sephadex™ LH-20 matrix.

22. A method according to claim 21 wherein the chromatographic step is carried out using a 2:1 mixture of chloroform/dichloromethane as solvent.

23. A method according to any one of the preceding claims wherein the first and second solvents are different, and wherein one of the first or second solvents is a solvent which is substantially more polar than the cannabinoid/cannabinoid acid which it is desired to purify, and the other solvent is a solvent which is substantially less polar than the cannabinoid/cannabinoid acid which it is desired to purify.

24. A method according to claim 23 wherein one of the solvents is an alcohol.

25. A method according to claim 24 wherein one of the solvents is methanol.

26. A method according to any one of claims 23 to 25 wherein one of the solvents is a straight or branched chain C5-C12 alkane.

27. A method according to claim 26 wherein one of the solvents is pentane.

28. A method according to claim 27 wherein one of the solvents is pentane and the other solvent is methanol.

29. A substantially pure preparation of Δ^9 tetrahydrocannabinolic acid (Δ^9 THCA) having a chromatographic purity of greater than 95%, more preferably greater than 96%, more preferably greater than 97% and most preferably greater than 98% by area normalisation of an HPLC profile.

30. A preparation according to claim 29 which is a pale yellow crystalline solid at room temperature.

31. A preparation according to claim 29 or claim 30 which comprises less than 2%, preferably less than 1.5%, most preferably 1% or less Δ^9 THC (w/w).

32. A preparation according to any one of claims 29 to 31 which comprises less than 2%, more preferably less than 1.5%, more preferably less than 1% and most preferably less than 0.5% CBD (w/w).

33. A preparation according to any one of claims 29 to 32 which comprises less than 2%, more preferably less than 1.5%, and most preferably less than 1% CBN (w/w).

34. A preparation according to any one of claims 29 to 33 which is obtainable from cannabis plant material using a method comprising:

i) preparing an extract of the cannabis plant material with 0.1% v/v acetic acid in hexane,
ii) filtering the resultant extract and removing solvent from filtrate by rotary evaporation to form an extract enriched in Δ^9 THCA,
iii) passing a solution of the resulting Δ^9 THCA enriched extract through a column packed with Sephadex-LH20™, eluting with 2:1 chloroform/dichloromethane,
iv) collecting Δ^9 THCA rich fractions eluted from the column and removing solvent by rotary evaporation,
v) re-dissolving the crude Δ^9 THCA obtained in step iv) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,

vi) re-dissolving the product of step v) in pentane, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation to produce Δ^9 THCA crystals.

35. A substantially pure preparation of Δ^9 tetrahydrocannabinolic acid (Δ^9 THCA) substantially as described herein and having an HPLC profile substantially as shown in Figure 2.

36. A substantially pure preparation of cannabidiolic acid (CBDA) having a chromatographic purity of greater than 90%, more preferable greater than 92% and most preferably greater than 94% by area normalisation of an HPLC profile.

37. A preparation according to claim 36 which is a pale yellow crystalline solid at room temperature.

38. A preparation according to claim 36 or claim 37 comprising 5% or less, preferably 4.5% or less, more preferably 4% or less, more preferably 3.5% or less and most preferably 3% or less CBD (w/w).

39. A preparation according to any one of claims 36 to 38 comprising less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% Δ^9 THCA (w/w).

40. A preparation according to any one of claims 36 to 40 comprising less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% Δ^9 THC (w/w)

41. A preparation according to any one of claims 36 to 40 which is obtainable from cannabis plant material using a method comprising:
i) preparing an extract of the cannabis plant material with 0.1% v/v acetic acid in hexane,
ii) filtering the resultant extract and removing solvent from filtrate by rotary evaporation to form an extract enriched in CBDA,
iii) passing a solution of the resulting CBDA enriched extract through a column packed with Sephadex-LH20™, eluting with 2:1 chloroform/dichloromethane,
iv) collecting CBDA rich fractions eluted from the column and removing solvent by rotary evaporation,
v) re-dissolving the crude CBDA obtained in step iv) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vi) re-dissolving the product of step v) in pentane, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation to produce CBDA crystals.

42. A substantially pure preparation of cannabidiolic acid (CBDA) substantially as described herein and having an HPLC profile substantially as shown in Figure 4.

43. A substantially pure preparation of Δ^9 tetrahydrocannabinol (Δ^9 THC) having a chromatographic purity of >99% by area normalisation of an HPLC profile.

44. A preparation according to claim 43 which is a semi-solid at room temperature.

45. A preparation according to claim 43 or claim 44 which comprises less 0.5%, preferably than 0.4%,

more preferably less than 0.2% and most preferably less than 0.1% CBD (w/w).

46. A preparation according to any one of claims 43 to 45 which comprises less than 0.5%, preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBN (w/w).

47. A preparation according to any one of claims 43 to 46 which is obtainable from cannabis plant material using a method comprising:

i) obtaining an ethanolic solution of a botanical drug substance from decarboxylated cannabis plant material,
ii) passing the solution obtained in step i) through a column of activated charcoal, and collecting the eluate,
iii) remove solvent from the eluate by rotary evaporation to give a Δ^9 THC enriched fraction,
iv) passing a solution of the resulting Δ^9 THC enriched extract through a column packed with Sephadex LH20, eluting with 2:1 chloroform/dichloromethane,
v) collecting Δ^9 THC rich fractions and removing solvent by rotary evaporation,
vi) re-dissolving the crude Δ^9 THC prepared in step v) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vii) re-dissolving the crude Δ^9 THC prepared in step vi) in pentane, removing insoluble residue by filtration and removing solvent from the filtrate by rotary evaporation to give a semi-solid preparation of Δ^9 THC.

48. A preparation according to claim 47 wherein the ethanolic solution of a botanical drug substance from decarboxylated cannabis plant material is obtained by a method comprising the following steps:

i) harvesting cannabis plant material,
ii) decarboxylation of the plant material,
iii) extraction with liquid carbon dioxide (CO_2), removal of CO_2 to recover crude extract,
iv) dissolution of crude extract in ethanol followed by chilling of the solution to precipitate unwanted waxes,
v) removal of unwanted waxy material by cold filtration.

49. A substantially pure preparation of Δ^9 tetrahydrocannabinol (Δ^9 THC) substantially as described herein and having an HPLC profile substantially as shown in Figure 7.

50. A substantially pure preparation of Δ^9 tetrahydrocannabivarin (Δ^9 THCV) having a chromatographic purity of greater than 95%, more preferable greater than 96%, more preferable greater than 97%, more preferable greater than 98%, and most preferable greater than 99% by area normalisation of an HPLC profile.

51. A preparation according to claim 50 which is a crystalline solid at room temperature.

52. A preparation according to claim 50 or claim 51 which comprises less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBD (w/w).

53. A preparation according to any one of claims 50 to 52 which comprises less than 2.0%, preferably less than 1.5%, more preferably less than 1.0% and most preferably 0.5% or less Δ^9 THC (w/w).

54. A preparation according to any one of claims 50 to 53 which comprises less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBN (w/w).

55. A preparation according to any one of claims 50 to 54 which is obtainable from cannabis plant material using a method comprising:
i) obtaining an ethanolic solution of a botanical drug substance from cannabis plant material,
ii) passing the solution obtained in step i) through a column of activated charcoal, and collecting the eluate,
iii) remove solvent from the eluate by rotary evaporation to give a Δ^9 THCV enriched fraction,
iv) passing a solution of the resulting Δ^9 THCV enriched extract through a column packed with Sephadex LH20, eluting with 2:1 chloroform/dichloromethane,
v) collecting Δ^9 THCV rich fractions and removing solvent by rotary evaporation,
vi) re-dissolving the crude Δ^9 THCV prepared in step v) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vii) re-dissolving the crude Δ^9 THCV prepared in step vi) in pentane, removing insoluble residue by filtration and removing solvent from the filtrate by rotary evaporation to give crystals of Δ^9 THCV.

56. A preparation according to claim 55 wherein the ethanolic solution of a botanical drug substance from decarboxylated cannabis plant material is obtained by a method comprising the following steps:

i) harvesting cannabis plant material,
ii) extraction with liquid carbon dioxide (CO_2), removal of CO_2 to recover crude extract,

iii) dissolution of crude extract in ethanol followed by chilling of the solution to precipitate unwanted waxes,
vi) removal of unwanted waxy material by cold filtration.

57. A substantially pure preparation of Δ^9 tetrahydrocannabivarin (Δ^9 THCV) substantially as described herein and having an HPLC profile substantially as shown in Figure 10.

58. A product enriched in cannabigerol (CBG) having a chromatographic purity of greater than 90%, preferably greater than 92% by area normalisation of an HPLC profile.

59. A product according to claim 58 which is an orange-yellow semi-solid at room temperature.

60. A product according to claim 58 or claim 59 which comprises less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBD (w/w).

61. A product according to any one of claims 58 to 60 which comprises less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably 0.1% or less Δ^9 THC (w/w).

62. A product according to any one of claims 58 to 61 which comprises less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably less than 0.1% CBD (w/w).

63. A product according to any one of claims 59 to 62 which is obtainable from cannabis plant material using a method comprising:
i) decarboxylating the cannabis plant material,
ii) preparing an extract of the decarboxylated cannabis plant material with hexane,
iii) filtering the resultant extract and removing solvent from filtrate by rotary evaporation to form an extract enriched in CBG,
iv) passing a solution of the resulting CBG enriched extract through a column packed with Sephadex-LH20™, eluting with 2:1 chloroform/dichloromethane,
v) collecting CBG rich fractions eluted from the column and removing solvent by rotary evaporation,
vi) re-dissolving the crude CBG obtained in step v) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vii) re-dissolving the product of step vi) in pentane, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation to produce a highly enriched CBG extract.

64. A product enriched in cannabigerol (CBG) substantially as described herein and having an HPLC profile substantially as shown in Figure 13.

65. A product enriched in cannabichromene (CBC) having a chromatographic purity of greater than 80%, more preferably greater than 85% by area normalisation of an HPLC profile.

66. A product according to claim 65 which is an orange-yellow semi-solid at room temperature.

67. A product according to claim 65 or claim 66 which comprises less than 5%, preferably less than 4%,

more preferably less than 3%, more preferably less than 2% and most preferably 1% or less CBD (w/w).

68. A product according to any one of claims 65 to 67 which comprises less than 2%, preferably less than 1.5%, more preferably less than 1.0%, more preferably less than 0.5% and most preferably 0.3% or less Δ^9 THC (w/w).

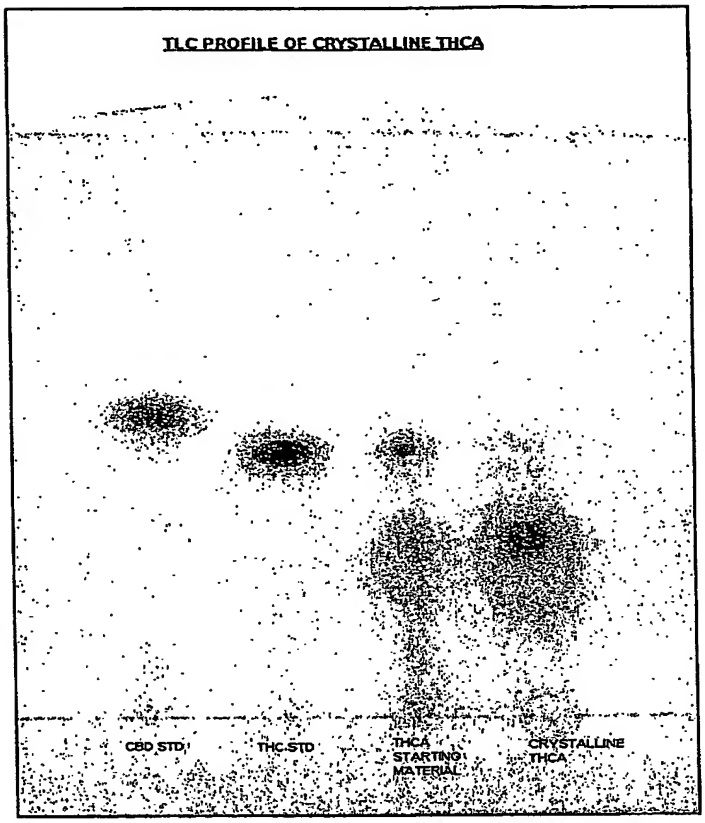
69. A product according to any one of claims 65 to 68 which comprises less than 1%, preferably less than 0.8%, more preferably less than 0.6%, more preferably less than 0.4%, more preferably less than 0.2% and most preferably 0.1% or less CBN (w/w).

70. A product according to any one of claims 65 to 69 which is obtainable from cannabis plant material using a method comprising:
i) decarboxylating the cannabis plant material,
ii) preparing an extract of the decarboxylated cannabis plant material with hexane,
iii) filtering the resultant extract and removing solvent from filtrate by rotary evaporation to form an extract enriched in CBC,
iv) passing a solution of the resulting CBC enriched extract through a column packed with Sephadex-LH20™, eluting with 2:1 chloroform/dichloromethane,
v) collecting CBC rich fractions eluted from the column and removing solvent by rotary evaporation,
vi) re-dissolving the crude CBC obtained in step v) in methanol, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation,
vii) re-dissolving the product of step vi) in pentane, removing insoluble residue by filtration and removing solvent from filtrate by rotary evaporation to produce a highly enriched CBC extract.

71. A product enriched in cannabichromene (CBC) substantially as described herein and having an HPLC profile substantially as shown in Figure 16.

Fig. 1.

TLC profile of G1 chemovar starting material and purified THCA



CHROMATOGRAPHIC CONDITIONS:

Stationary phase: SIL G/UV_{254}
Mobile phase: Hexane: diethyl ether 80:20
Development distance: double development.
Visualisation: 0.1% w/v Fast Blue B salt in water

Standards 1 mg/ml CBD (BN 10601/C) in MeOH
5 ul applied to TLC plate.
1 mg/ml d9 THC (BN 10601/B) in MeOH
5 ul applied to TLC plate.

Samples 1 mg/ml THCA STARTING MATERIAL in MeOH
5 ul applied to TLC plate.
1 mg/ml CRYSTALLINE THCA in MeOH
5 ul applied to TLC plate.

HPLC profiles of G1 chemovar starting material and purified d9 THCA

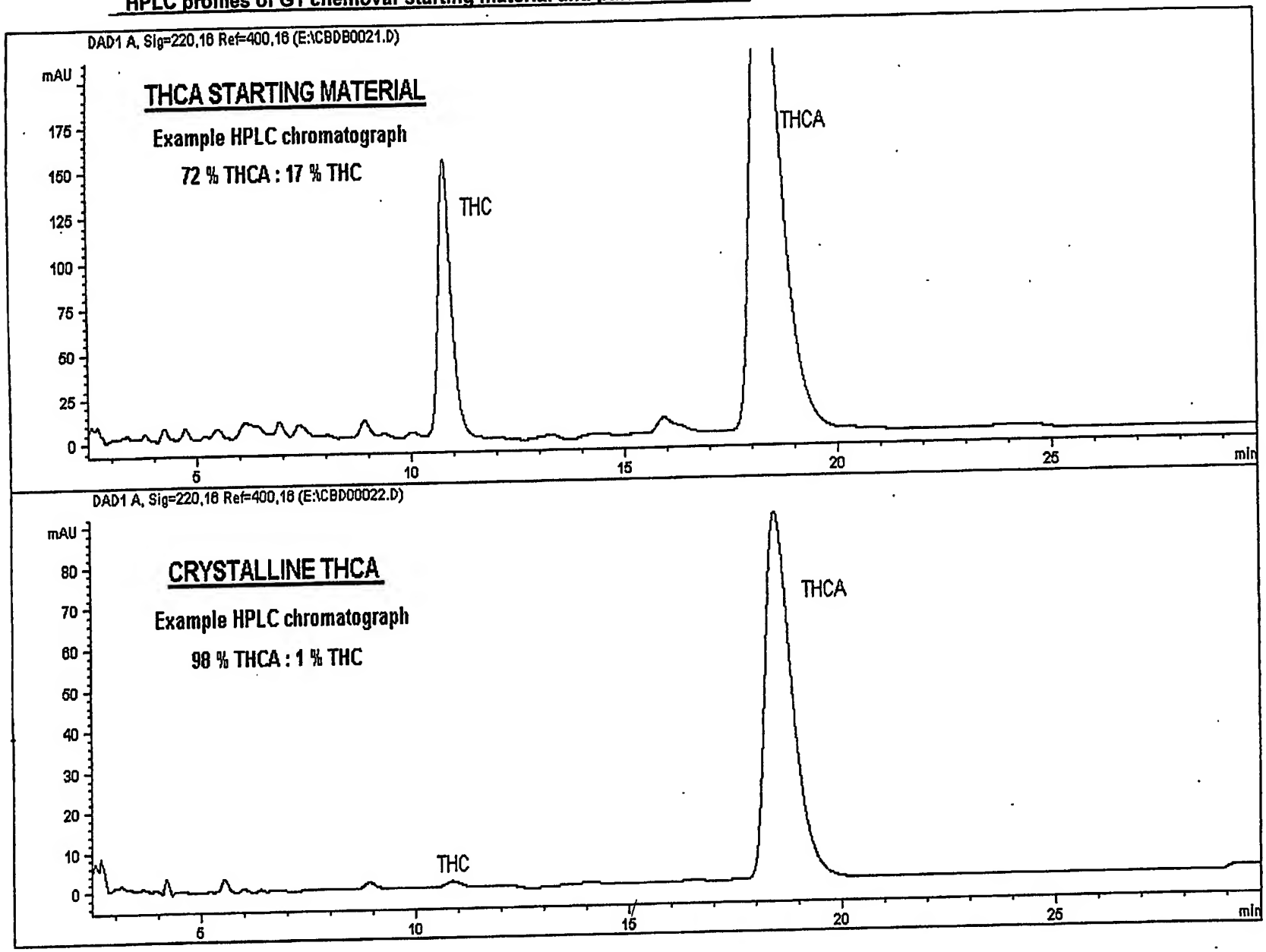


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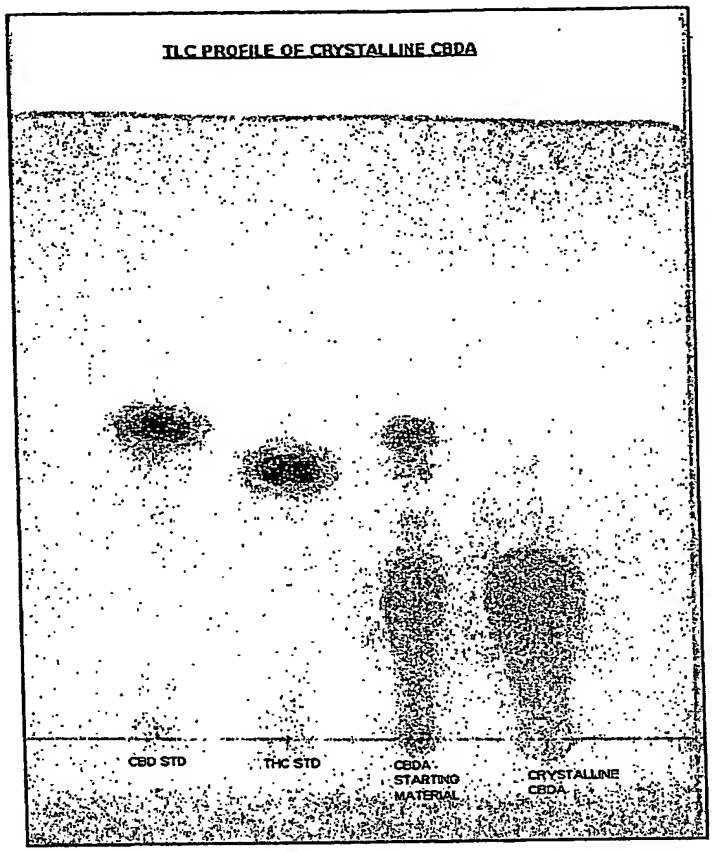
Fig. 2.

Fig. 3.

TLC profile of G5 chemovar starting material and purified CBDA



CHROMATOGRAPHIC CONDITIONS:

Stationary phase: SIL G/UV_{254}
Mobile phase: Hexane: diethyl ether 80:20
Development distance: double development.
Visualisation: 0.1% w/v Fast Blue B salt in water

Standards 1 mg/ml CBD (BN 10601/C) in MeOH
5 ul applied to TLC plate.
1 mg/ml d9 THC (BN 10601/B) in MeOH
5 ul applied to TLC plate.

Samples 1 mg/ml CBDA STARTING MATERIAL in MeOH
5 ul applied to TLC plate.
1 mg/ml CRYSTALLINE CBDA in MeOH
5 ul applied to TLC plate.

HPLC profiles of G5 chemovar starting material and purified CBDA

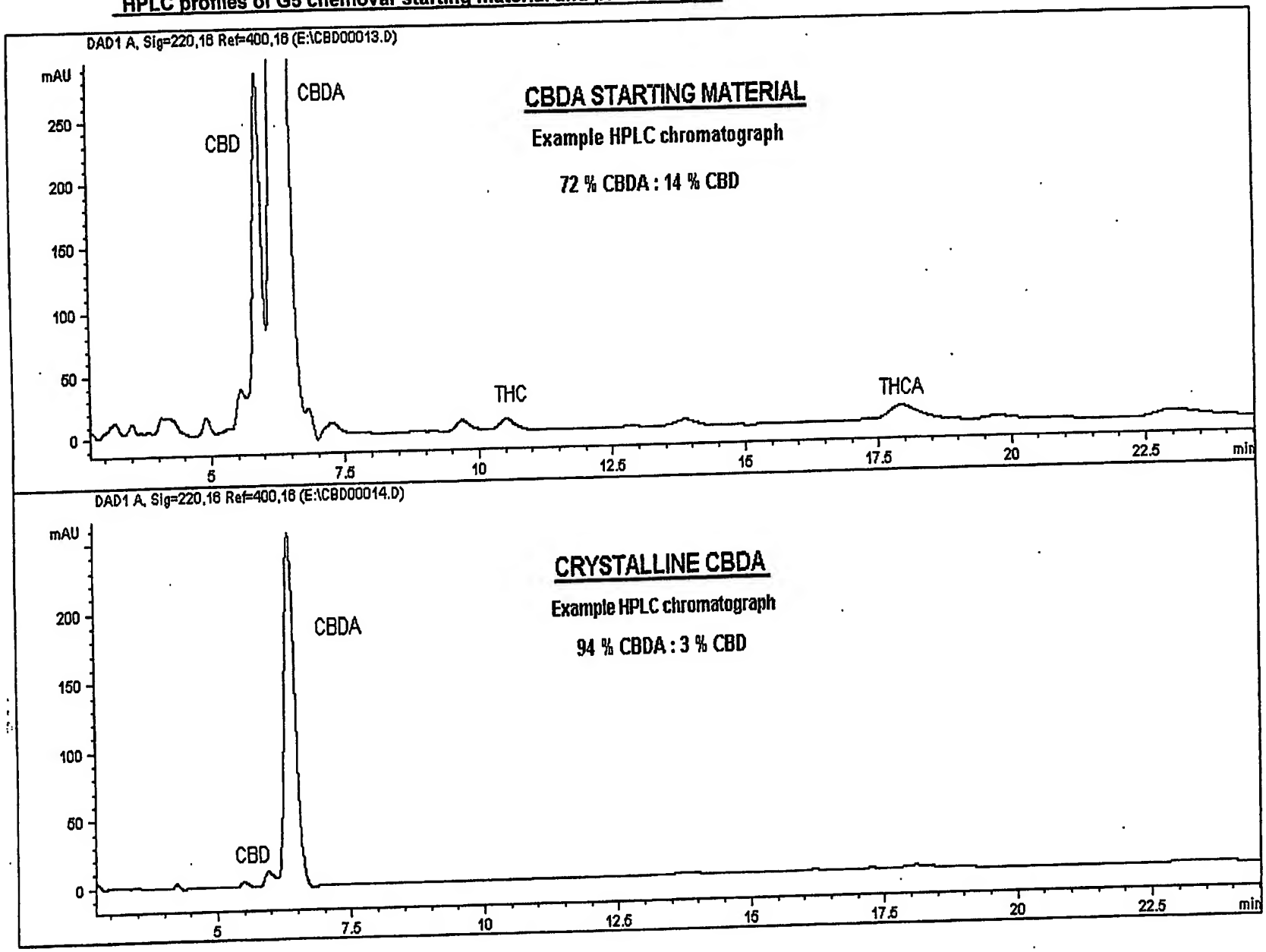


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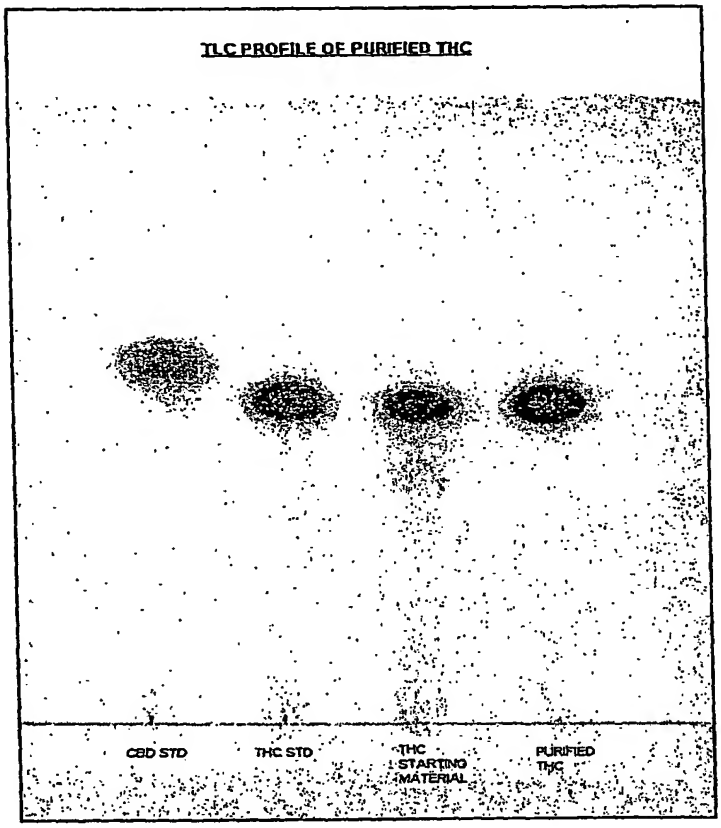
Fig. 4.

Fig. 5. 5/17

TLC profiles of BDS starting material and purified d9 THC



CHROMATOGRAPHIC CONDITIONS:

Stationary phase: SIL G/UV_{254}
Mobile phase: Hexane: diethyl ether 80:20
Development distance: double development.
Visualisation: 0.1% w/v Fast Blue B salt in water

Standards 1 mg/ml CBD (BN 10601/C) in MeOH
5 ul applied to TLC plate.
1 mg/ml d9 THC (BN 10601/B) in MeOH
5 ul applied to TLC plate.

Samples 1 mg/ml THC STARTING MATERIAL in MeOH
5 ul applied to TLC plate.
1 mg/ml PURIFIED THC in MeOH
5 ul applied to TLC plate.

HPLC profiles of BDS starting material and purified d9 THC

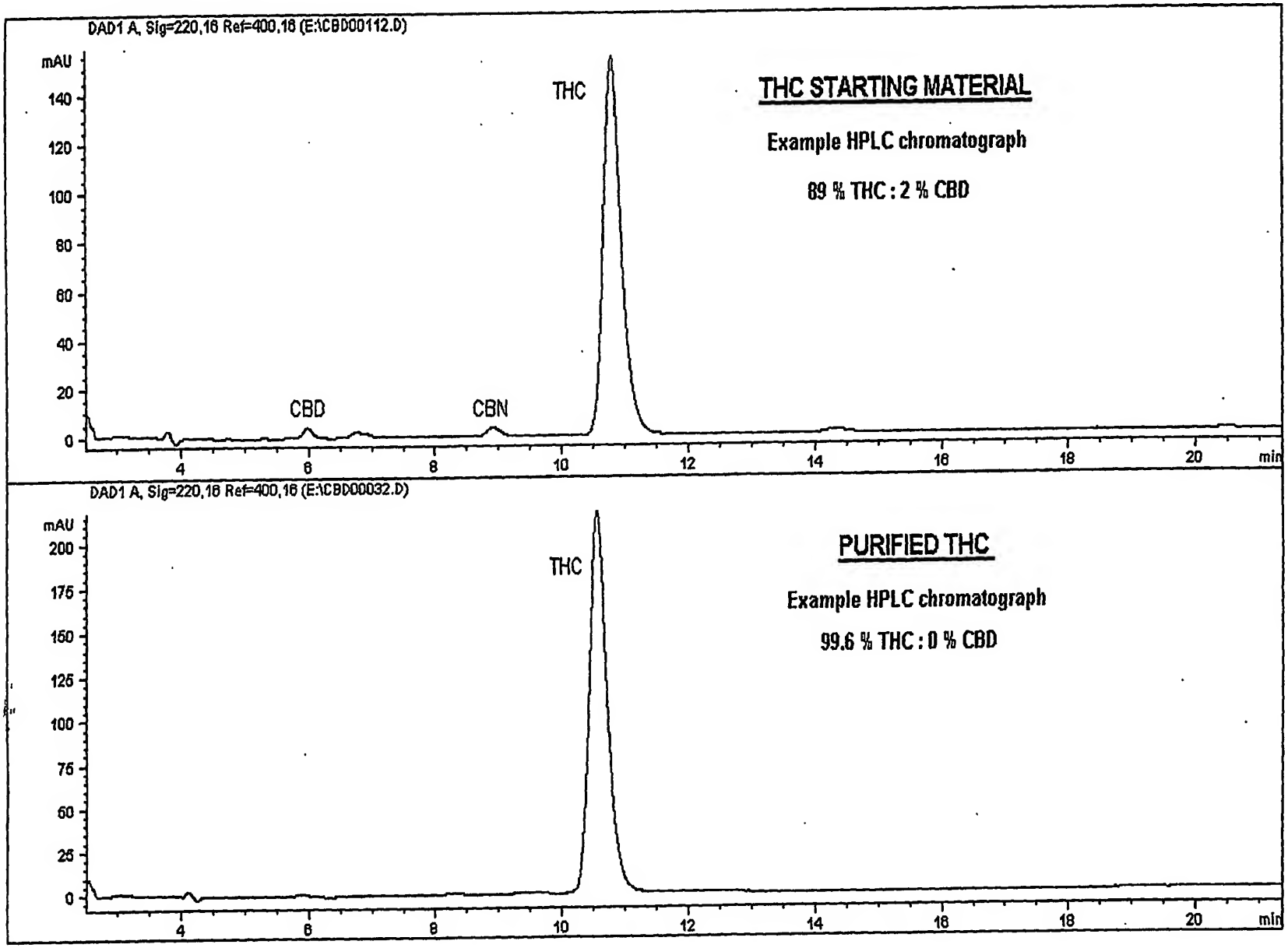


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Fig. 6.

Fig. 7.

Comparison of HPLC profiles of commercially available d9 THC standard (ex Sigma) and in house purified d9 THC

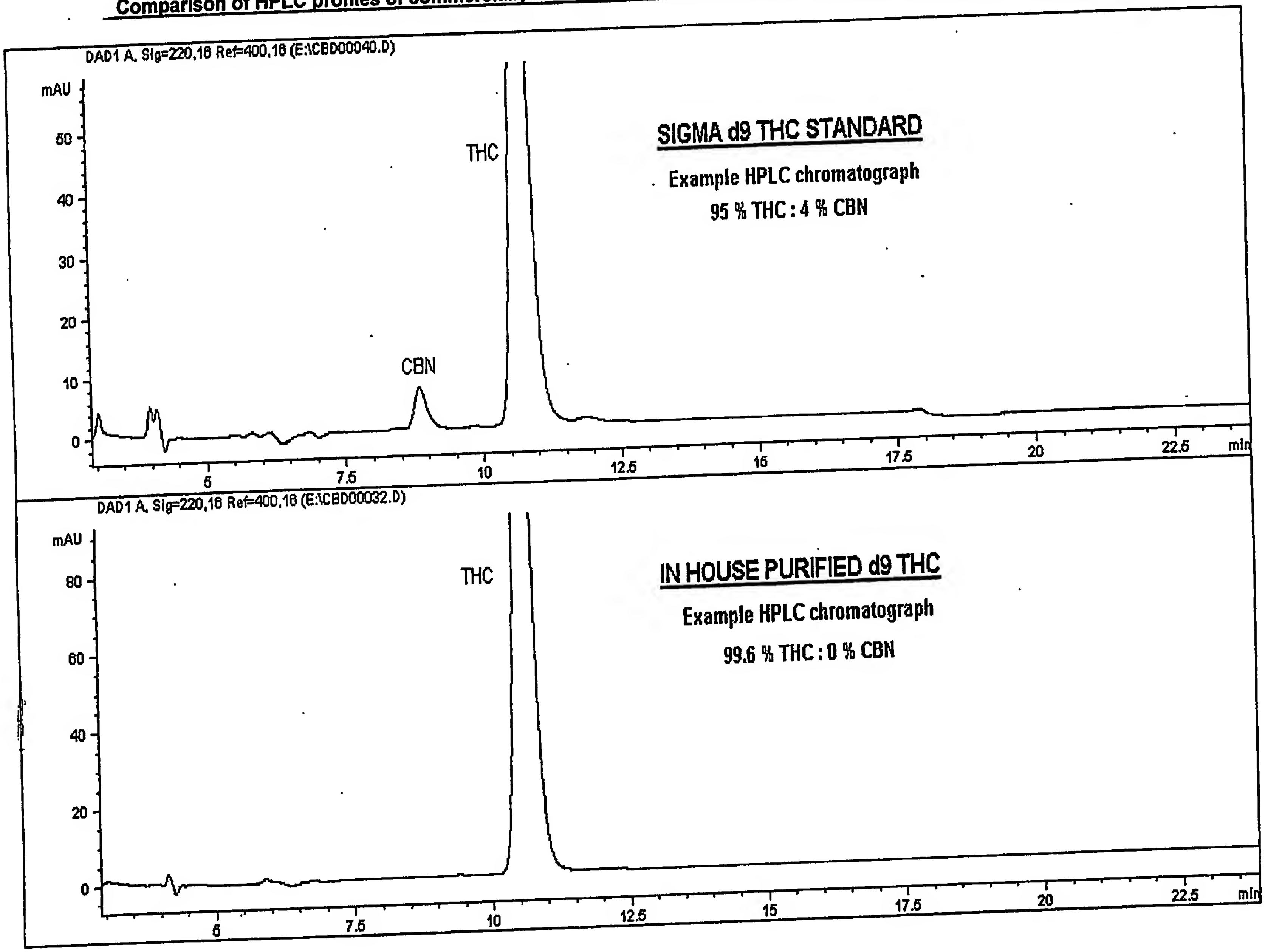


Fig-8

GC profiles of BDS starting material and purified d9 THC

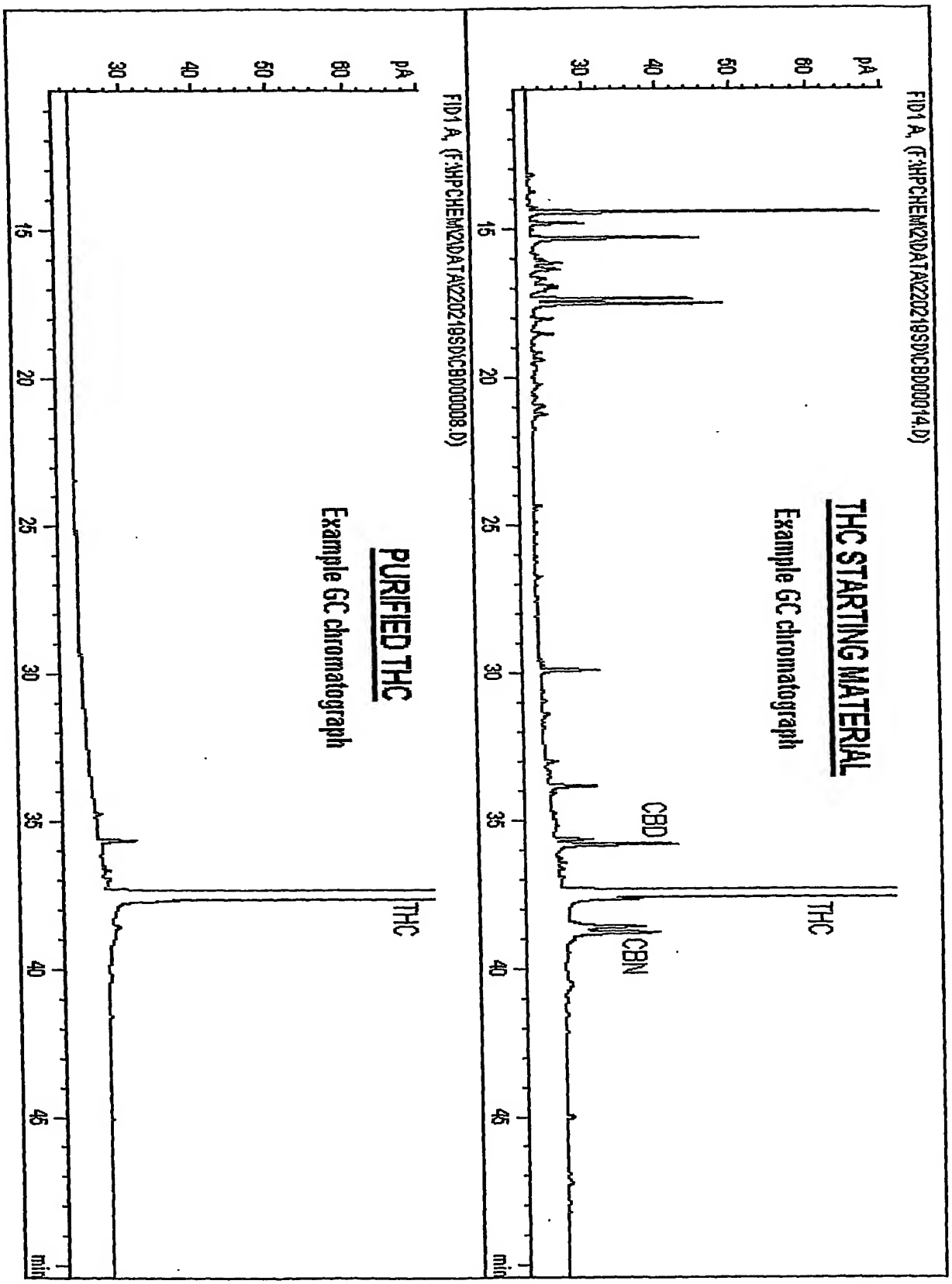
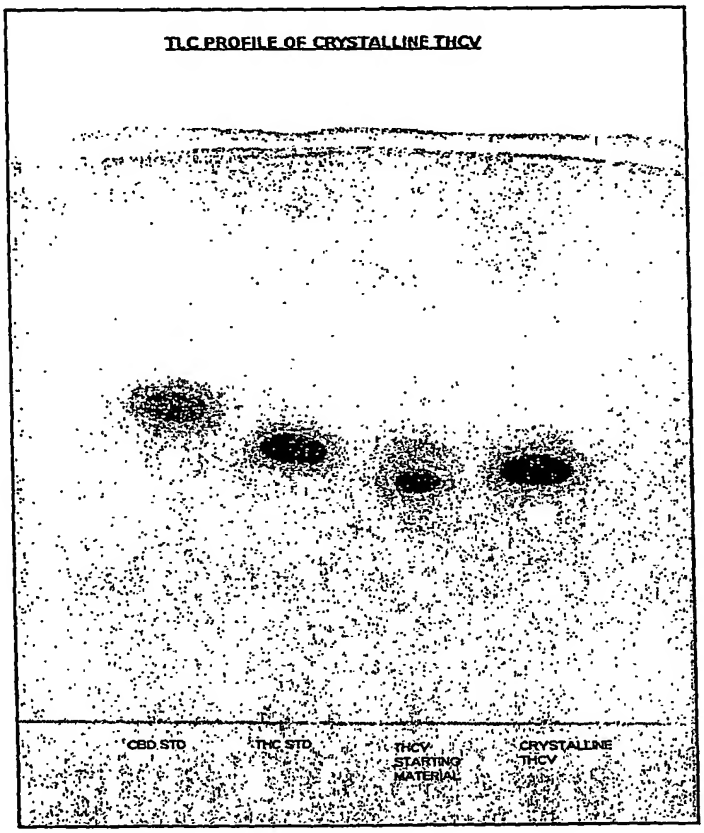


Fig. 9.

TLC profiles of BDS starting material and purified d9 THCV



CHROMATOGRAPHIC CONDITIONS:

Stationary phase: SIL G/UV_{254}
Mobile phase: Hexane: diethyl ether 80:20
Development distance: double development.
Visualisation: 0.1% w/v Fast Blue B salt in water

Standards 1 mg/ml CBD (BN 10601/C) in MeOH
5 ul applied to TLC plate.
1 mg/ml d9 THC (BN 10601/B) in MeOH
5 ul applied to TLC plate.

Samples 1 mg/ml THCV STARTING MATERIAL in MeOH
5 ul applied to TLC plate.
1 mg/ml CRYSTALLINE THCV in MeOH
5 ul applied to TLC plate.

Hplc profiles of BDS starting material and purified d9 THCV

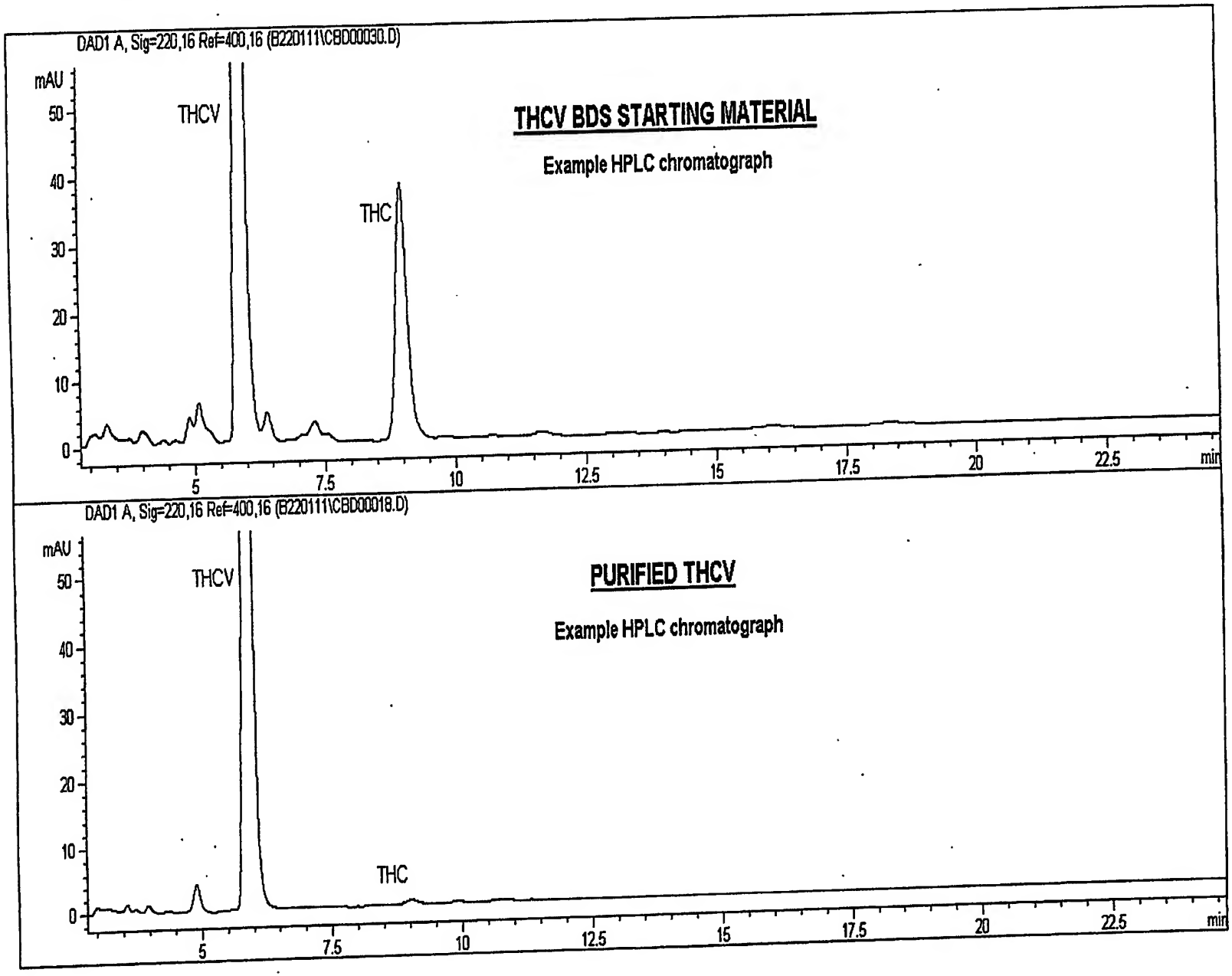


Fig. 10

GC profiles of BDS starting material and purified d9 THCV

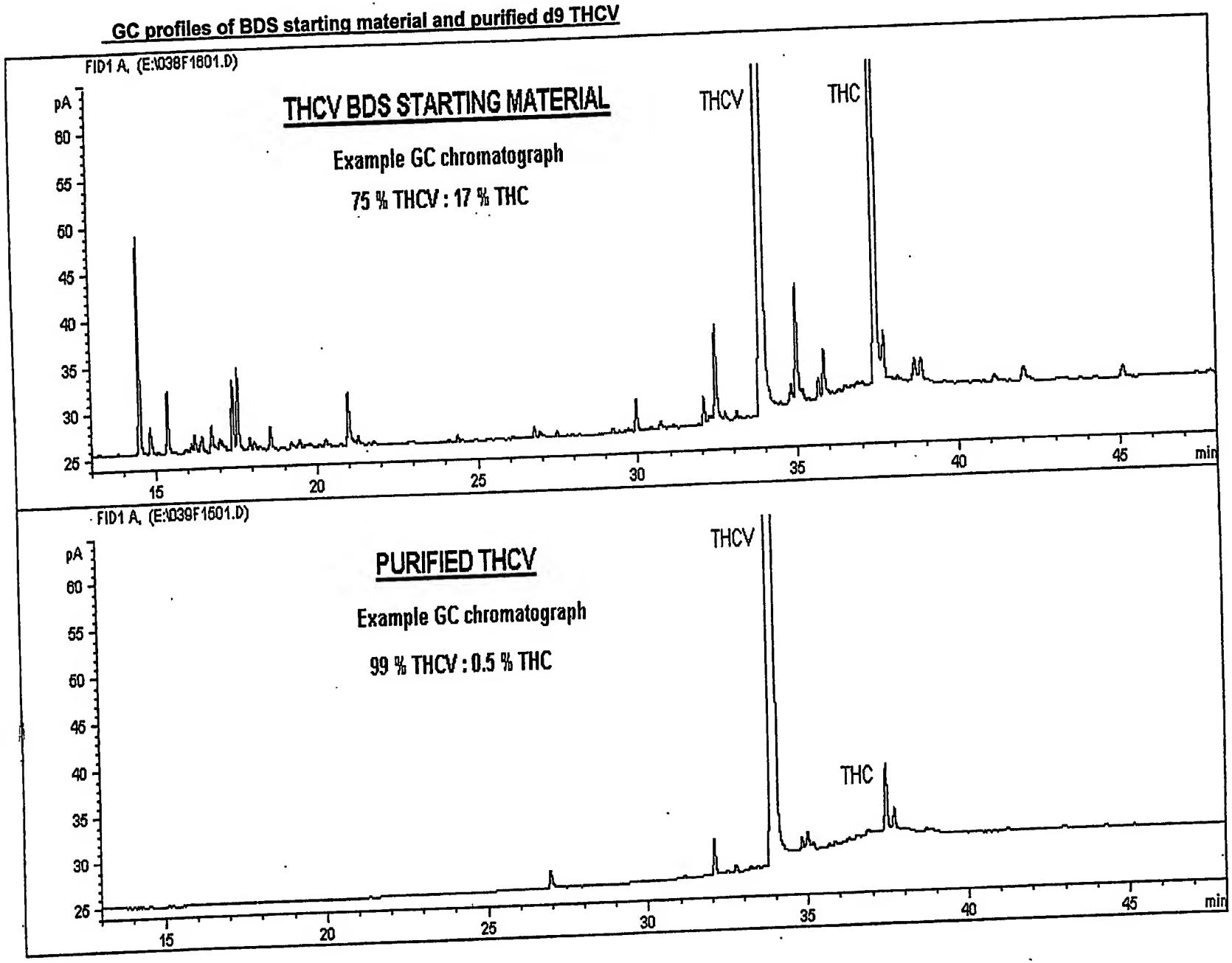


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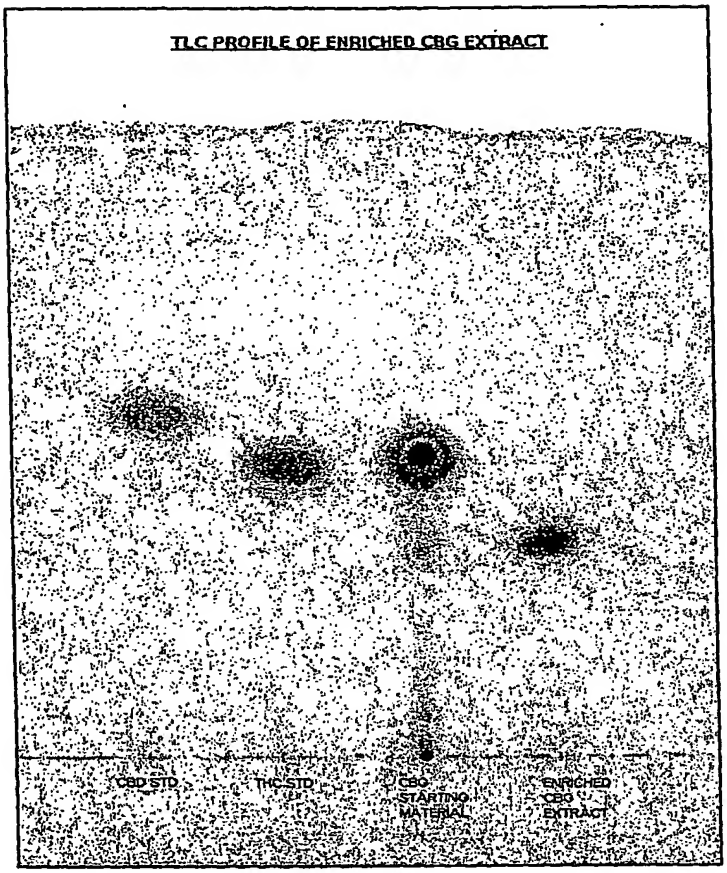
Fig. 11

Fig. 12

TLC profiles of G41chemovar starting material (post decarboxylation) and enriched CBG extract.



CHROMATOGRAPHIC CONDITIONS:

Stationary phase: SIL G/UV_{254}
Mobile phase: Hexane: diethyl ether 80:20
Development distance: double development.
Visualisation: 0.1% w/v Fast Blue B salt in water

Standards 1 mg/ml CBD (BN 10601/C) in MeOH
5 ul applied to TLC plate.
1 mg/ml d9 THC (BN 10601/B) in MeOH
5 ul applied to TLC plate.

Samples 1 mg/ml CBG STARTING MATERIAL in MeOH
5 ul applied to TLC plate.
1 mg/ml ENRICHED CBG EXTRACT in MeOH
5 ul applied to TLC plate.

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HPLC profiles of G41 chemovar starting material, pre and post decarboxylation, and enriched CBG extract

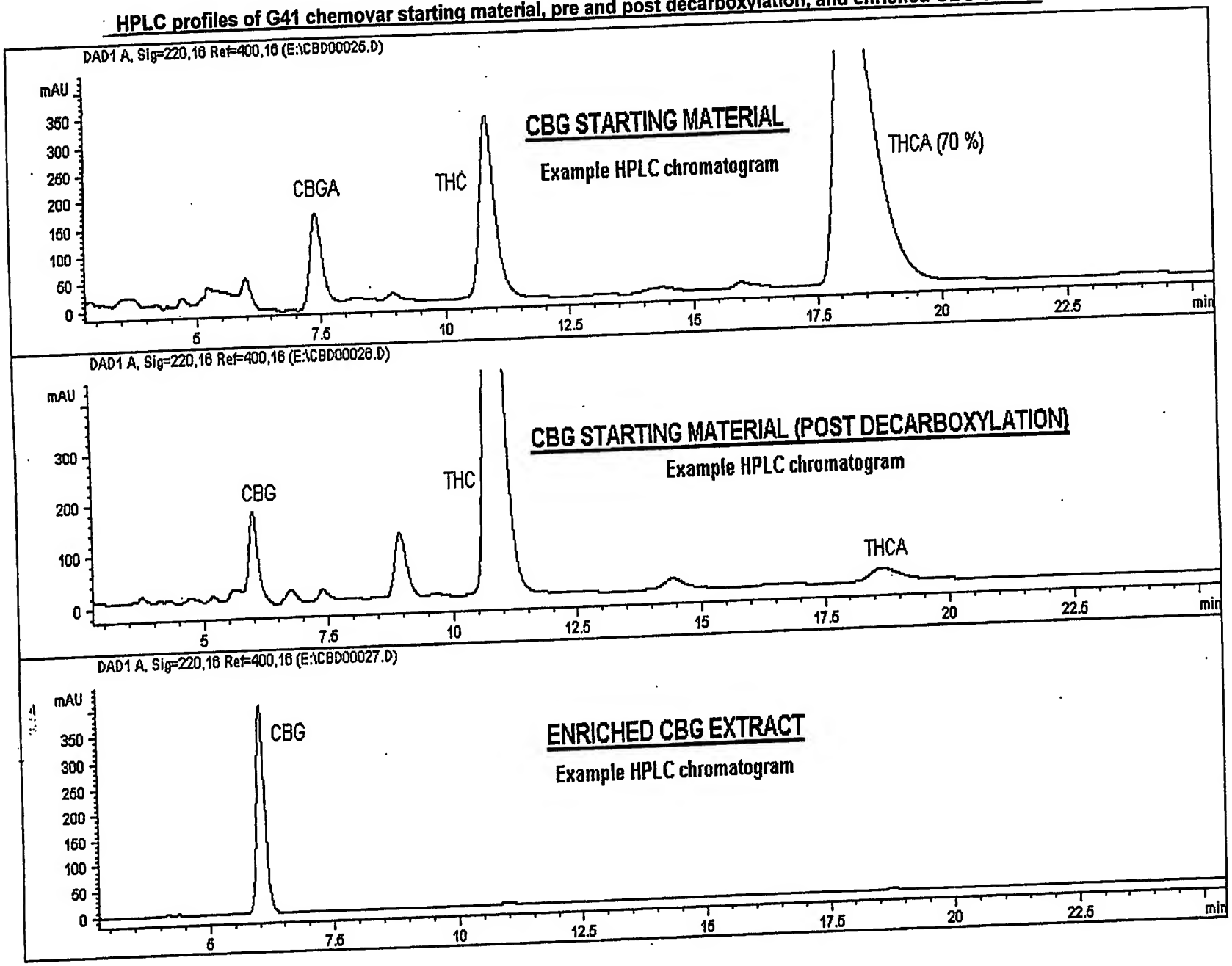


Fig. 13

Fig. 14

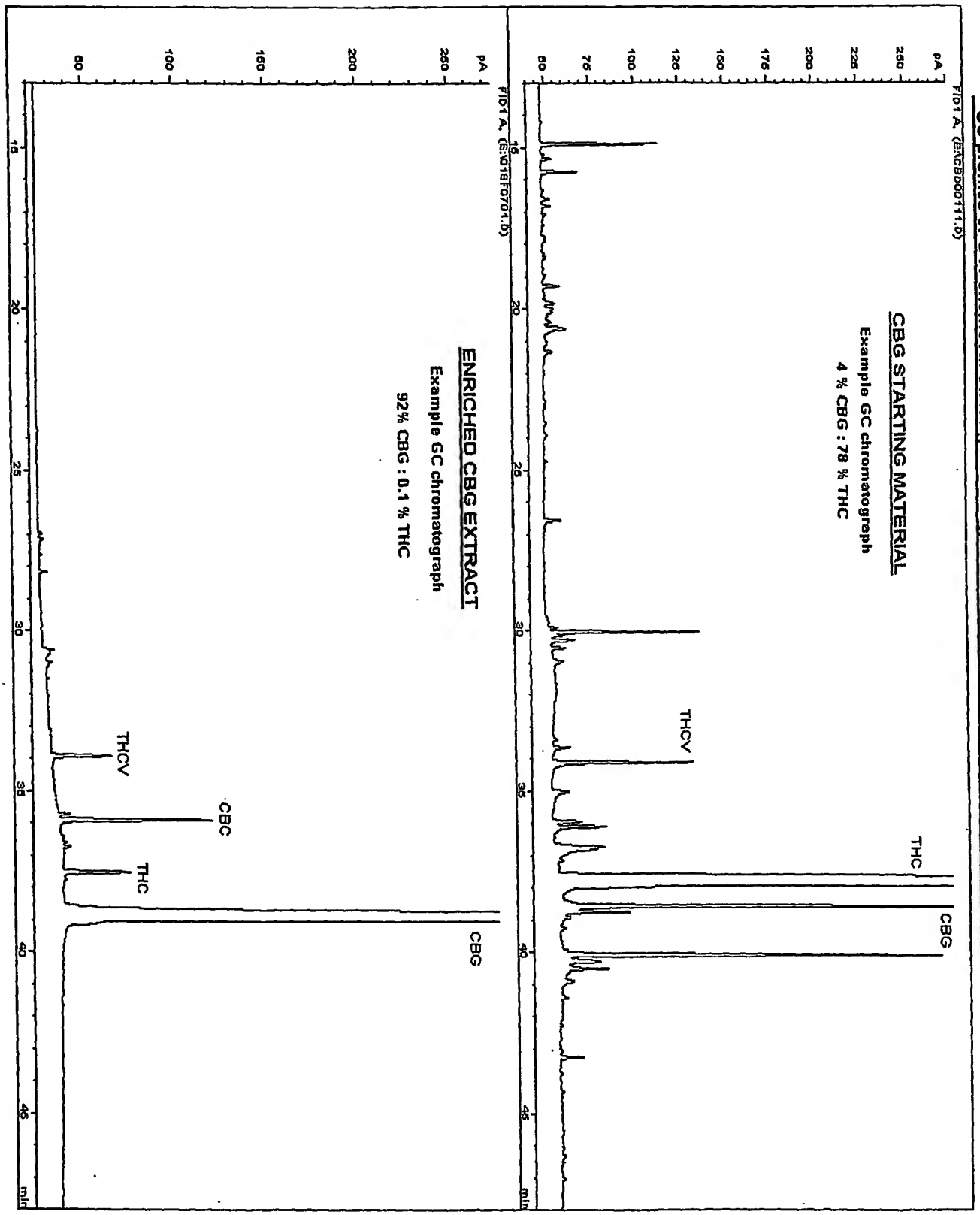
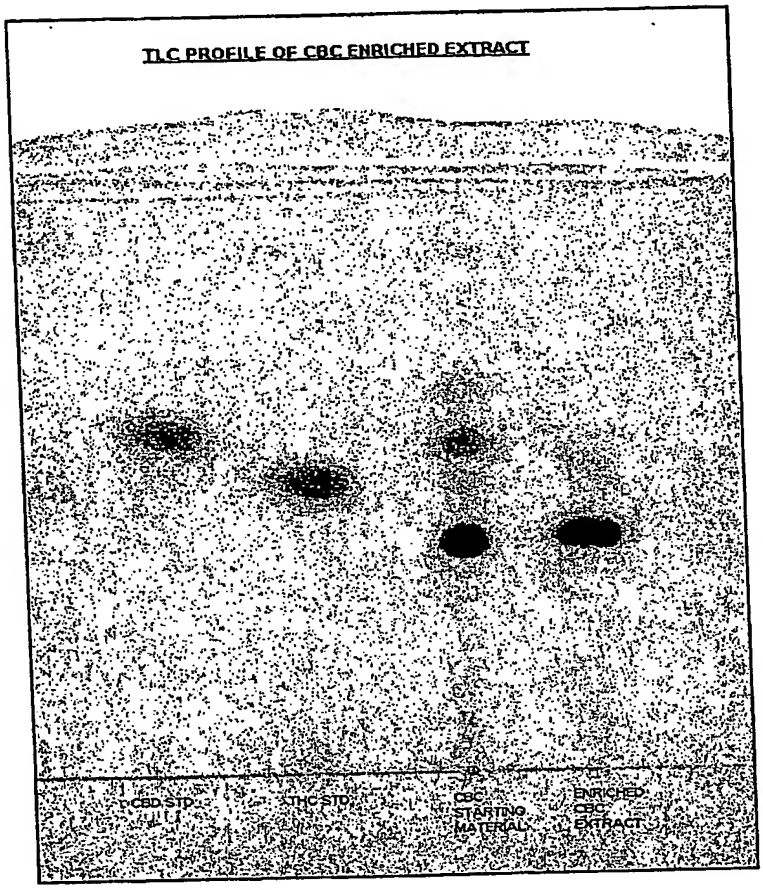

GC profiles of G41 chemovar starting material (post decarboxylation) and enriched CBG extract.
CBG STARTING MATERIAL
Example GC chromatograph
4 % CBG : 78 % THC
THC
CBG
THCV
ENRICHED CBG EXTRACT
Example GC chromatograph
92% CBG : 0.1 % THC
CBG
CBC
THCV
THC
pA
min

Fig. 15

TLC profiles of G80 chemovar starting material (post decarboxylation) and enriched CBC extract.



CHROMATOGRAPHIC CONDITIONS:

Stationary phase: SIL G/UV_{254}
Mobile phase: Hexane: diethyl ether 80:20
Development distance: double development.
Visualisation: 0.1% w/v Fast Blue B salt in water

Standards 1 mg/ml CBD (BN 10601/C) in MeOH
5 ul applied to TLC plate.
1 mg/ml d9 THC (BN 10601/B) in MeOH
5 ul applied to TLC plate.

Samples 1 mg/ml CBC STARTING MATERIAL in MeOH
5 ul applied to TLC plate.
1 mg/ml ENRICHED CBC EXTRACT in MeOH
5 ul applied to TLC plate.

HPLC profiles of G80 chemovar starting material, pre and post decarboxylation, and enriched CBC extract.

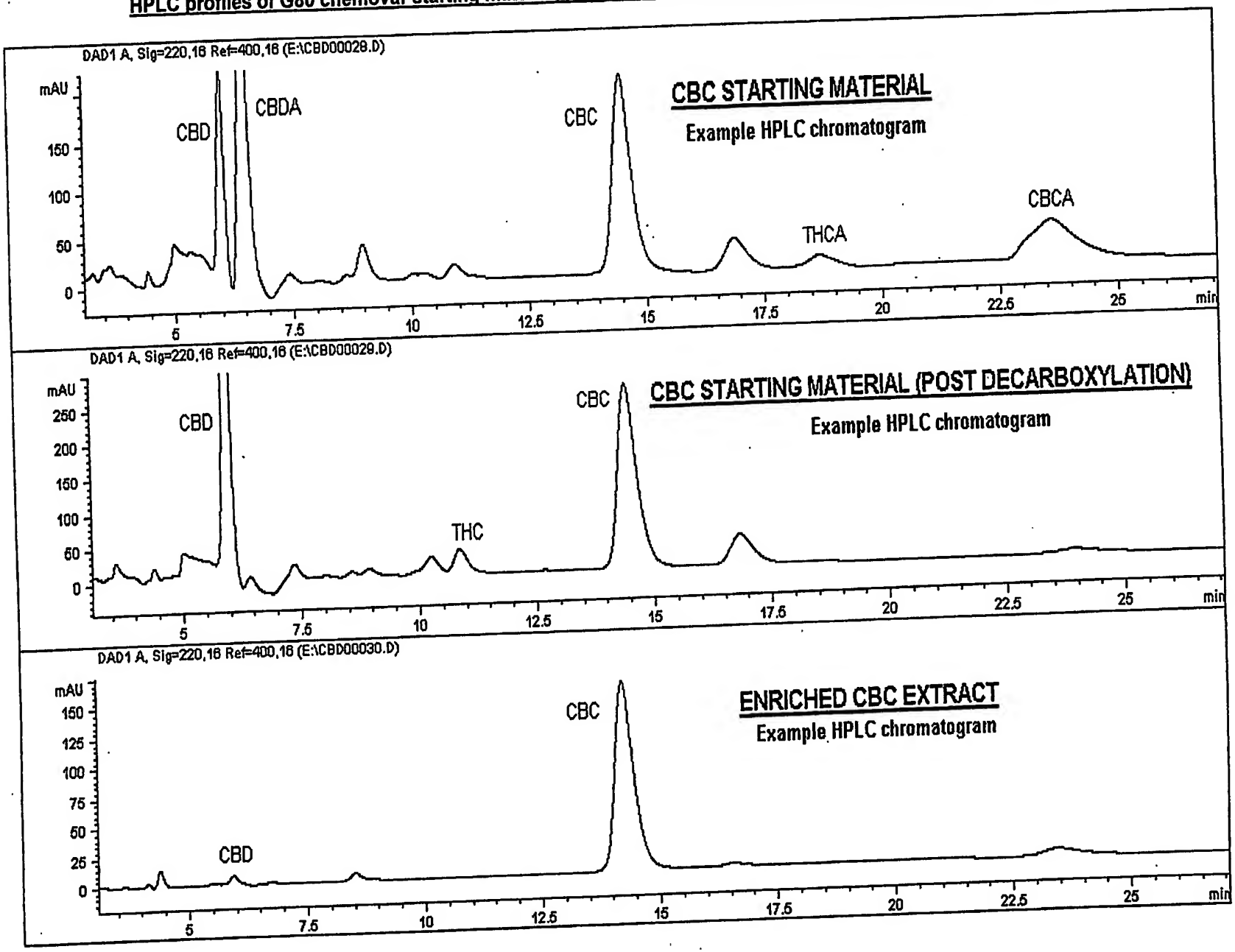


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Fig. 16

Fig. 17

GC profiles of G80 chemovar starting material (post decarboxylation) and enriched CBC extract.